“ HISTOMORPHOLOGICAL PROFILE OF ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING”

DISSERTATION SUBMITTED FOR

M.D. DEGREE EXAMINATION

BRANCH III PATHOLOGY

OF

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI

APRIL -2013

CERTIFICATE

This is to certify that the Dissertation **"HISTOMORPHOLOGICAL PROFILE OF ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING"** presented herein by **Dr. J.JOHNSY MERLA** is an original work done in the Department of Pathology, Tirunelveli Medical College Hospital, Tirunelveli for the award of Degree of M.D. (Branch III) Pathology under my guidance and supervision during the academic period of 2010 - 2013.

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I hereby certify that this work embodied in the dissertation entitled **"HISTOMORPHOLOGICAL PROFILE OF ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING"** is a record of work done by **Dr.J.Johnsy Merla,** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course in the period 2010-2013. This work has not formed the basis for any previous award of any degree.

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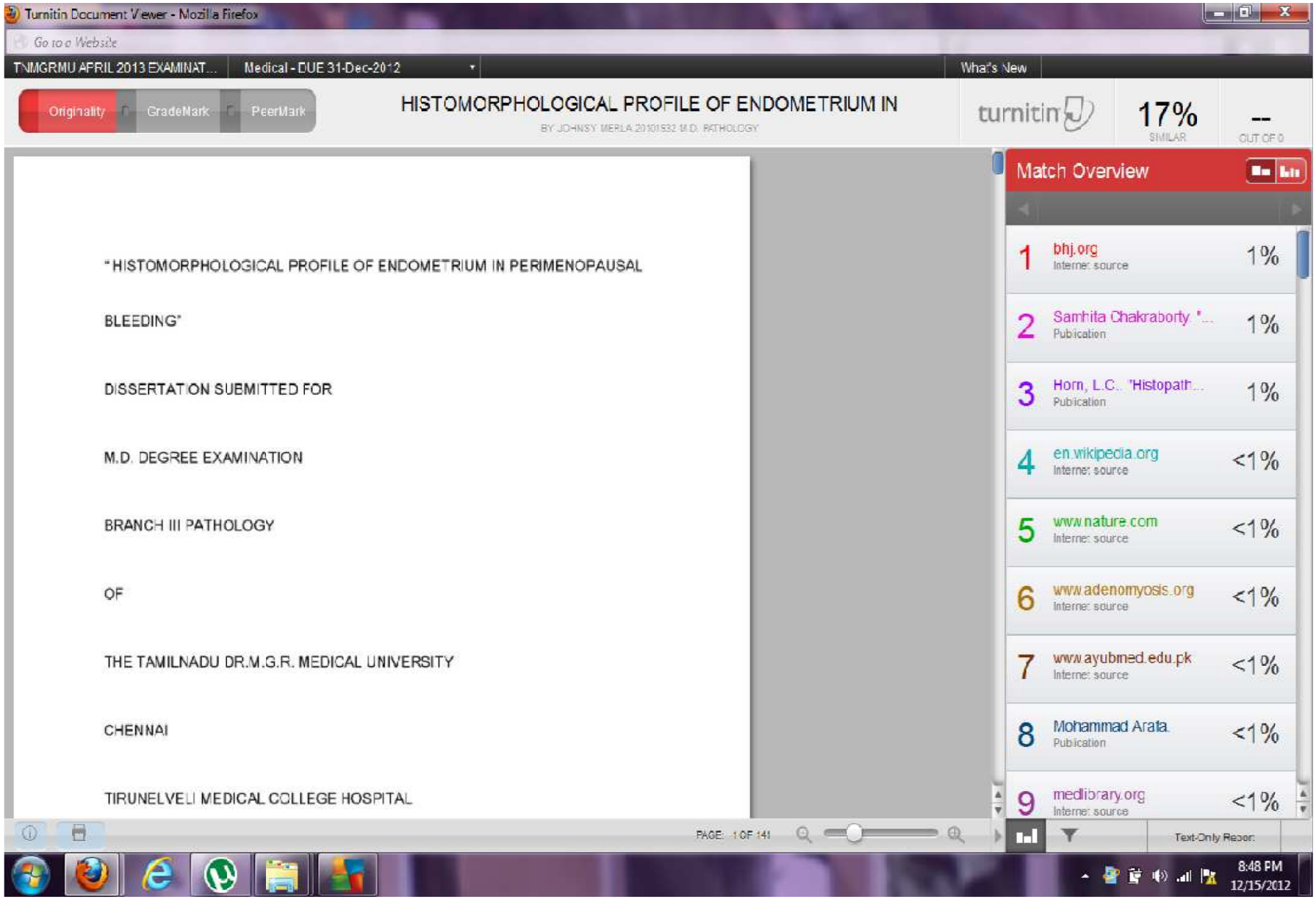
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I solemnly declare that the dissertation titled **"HISTOMORPHOLOGICAL PROFILE OF ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING"** is done by me at Tirunelveli Medical College hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R.Medical University towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) in Pathology.

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ABBREVIATIONS

AUB	:	Abnormal Uterine Bleeding
DAB	:	Diaminobenzidine
D&C	:	Dilatation and Curettage
DNA	:	De oxy ribonucleic Acid
DPX	:	Di-N-Butyle Phthalate in Xylene
DUB	:	Dysfunctional Uterine Bleeding
ER	:	Estrogen Receptor
ERα	:	Estrogen Receptor α isoform
ERβ	:	Estrogen Receptor β isoform
ERE	:	Estrogen Response Elements
FSH	:	Follicle Stimulating Hormone
HRP	:	Horse Radish Peroxidase
IHC	:	Immunohistochemistry
LH	:	Luteinizing Hormone
PR	:	Progesterone receptor
PRA	:	Progesterone Receptor A isoform
PRB	:	Progesterone Receptor B isoform
PRE	:	Progesterone Response Elements
WHO	:	World Health Organisation

CONTENTS

INTRODUCTION

Perimenopausal bleeding is one of the commonest conditions for which patients seek advice in the gynaecological outpatient department. The prevalence increases with age, peaking just prior to menopause. Because most cases are associated with anovulatory menstrual cycles, perimenopausal women are particularly vulnerable. Throughout the perimenopausal transition, there is a significant incidence of DUB due to anovulation[1]. Perimenopause is the period 2-8 years preceding the menopause and 1 year after final menses (WHO).However a better definition is the phase preceeding the onset of menopause, occurring around the age of 40-50 years (beginning at age 47.5, lasting for 4 years) during which the regular menstrual cycle of a woman transitions to a pattern of irregular cycles.[2]

Perimenopausal bleeding refers to the symptoms of excessive, unexpected, prolonged, or acyclic bleeding, regardless of the diagnosis or cause [3].With medical advancements and the increasing awareness about gynaecological problems, most women gain access to most of the diagnostic and therapeutic modalities. Endometrial biopsy is relatively simple, accurate and inexpensive. The only disadvantage of the endometrial biopsy is that, it is an invasive procedure. The main reason for obtaining endometrial histology in perimenopausal patients with bleeding is to exclude the presence of endometrial hyperplasia or

carcinoma of endometrium[4]. Both typical hyperplasia and atypical hyperplasia may regress spontaneously over months or few years. However, atypical hyperplasia is a precancerous condition that may progress to malignancy and best treated by surgery with hysterectomy. Hyperplasia without atypia regresses spontaneously after D&C or progestin treatment. In patients with atypical hyperplasia, if conserving the uterus is considered, a trial of hormonal treatment may be given. Analysis of steroid hormone receptors play an important role in patients with perimenopausal bleeding to predict the response to hormonal therapy[4]. Currently, the steroid hormone receptor status of carcinoma of the endometrium has been demonstrated to be prognostically important.

In this study of 200 cases an attempt is made to evaluate the histomorphological profile of endometrium in perimenopausal bleeding, and the immunohistochemical expression of ER and PR in endometrial hyperplasias.

AIMS AND OBJECTIVES

1. To evaluate the incidence of perimenopausal bleeding in our institution.
2. To identify the distribution of cases among various age groups.
3. Analysing the histomorphological pattern of endometrium in perimenopausal bleeding.
4. To evaluate the expression of ER and PR in endometrial hyperplasias by immunohistochemical method.

REVIEW OF LITERATURE

"The term "menstruation" arose from "menstruus" a latin word meaning "monthly". The literature on the etiology of uterine bleeding disorders dates back to 1846-53, when French surgeon Robert (1846) Robin and Nelatan (1853) observed that hyperplasia of the endometrium, is one of the common cause[5].

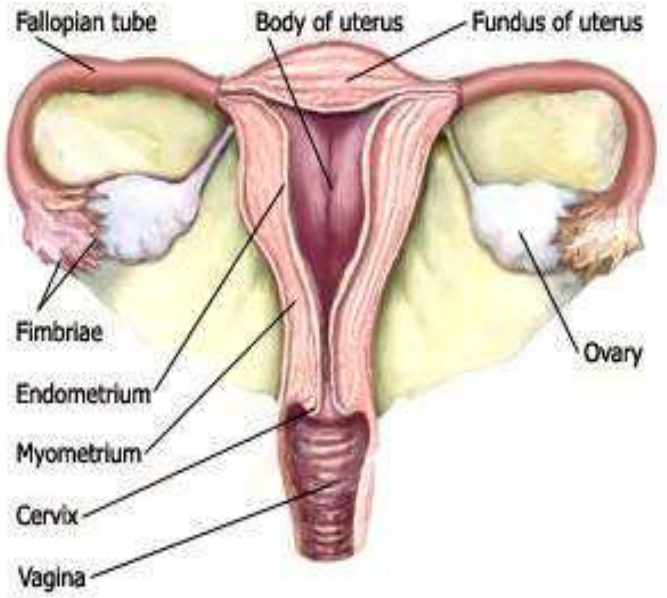


Figure 1: Schematic representation of the female genital tract.

PHYSIOLOGY OF MENSTRUATION:

As perimenopausal bleeding is a hormonal disorder, knowledge of the normal hormonal control mechanism of menstruation is useful.

The Phenomena of Menstruation:

Menstruation is defined as a 'periodic and cyclical shedding of the progestational endometrium accompanied by the loss of blood' during the reproductive age between menarche and menopause[6,7]. The normal menstrual cycle occurs approximately at 28 day intervals with a range of

21 - 35 days, the flow lasting for 4 ± 2 days, and the average blood loss is 40 ± 20 ml.

The menstrual cycle is under complex hormonal control and the morphology of endometrium closely reflects the endocrine status and the interplay between ovarian hormones. The cyclical endocrine activity of the ovary and the hypothalamic-pituitary axis determines the phases of a normal menstrual cycle[8].

The normal human menstrual cycle can be divided into two segments[9]

- The ovarian cycle
- The uterine cycle

The ovarian cycle is further divided into:

- Follicular phase
- Ovulation
- Luteal phase

The uterine cycle is divided into proliferative and secretory phases. The four major hormones that are involved in the control of menstrual cycle and measured in peripheral blood are:

- Follicular stimulating hormone (FSH)
- Luteinizing hormone (LH)
- Estrogen
- Progesterone

Their secretion pattern is inter-related and it reflects the cyclic patterns of hypothalamic activity. Starting at menarche, the uterus undergoes monthly cyclic changes caused by differential production and secretion of the ovarian hormones, estrogen and progesterone (Fig. 2).

OVARIAN CYCLE

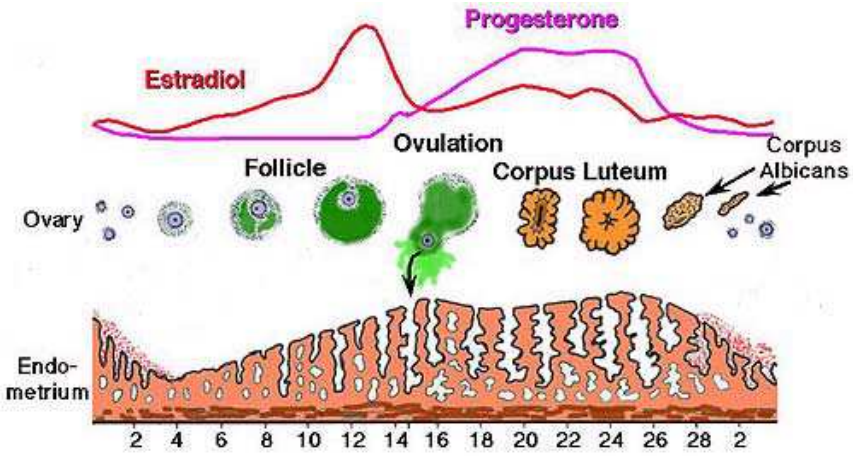


Figure 2 : Changes in the endometrium in relation to the changes in the ovarian cycle.

1. The follicular phase:

During the follicular phase,a sequence of events takes place as a result of which mature follicles are produced.This process in which the follicle matures through the stages of primordial follicle to the stages of preantral, antral, and preovulatory follicles[10] requires a coordinated action of hormones on the ovarian follicles.This occurs over a span of 10-14 days. Variations in length of follicular phase is the cause for most of the variations in total cycle length.

2. Ovulation:

The preovulatory follicle, provides its own ovulatory stimulus through the secretion of estradiol. Even in the same woman,the timing of ovulation varies from cycle to cycle. It is estimated that the time of ovulation is 10-12 hours after the peak level of LH and 24-36 hours after the peak estradiol levels. 34-36 hours prior to rupture of the ovarian follicle, the LH surge occurs and it is the most reliable indicator of ovulation. Maintenance of LH concentration for a threshold period of 14-27 hours helps in full maturation of the oocyte. LH surge usually lasts for 48-50 hours.

3. Luteal phase:

Before follicular rupture and the extrusion of the ovum, the granulosa cells begin to increase in size and has a characteristic vacuolated appearance. This is associated with the formation of the corpus luteum, which has a characteristic yellow colour due to the deposition of yellow pigment, lutein, which derives its name from the process of luteinization. The duration from ovulation to the menstrual onset constitutes the luteal phase , with an average length of 14 days.

UTERINE CYCLE:

The mucosal lining of the uterus, the endometrium, is composed of the glands and the stroma. The endometrium is composed of two layers.[9]

The Functionalis:

This is the superficial two thirds of the endometrium that proliferates and is shed with each menstrual cycle if pregnancy does not occur. The functionalis may be differentiated into superficial compacta and the underlying spongiosa, in the second half of the menstrual cycle.

The basalis:

This is the deepest layer of the endometrium which does not undergo cyclical changes observed in the functional layer. This basal layer persists after menstruation and regenerates the functional layer.

The endometrium varies in thickness throughout the cycles[11].

- At menstruation - 0.5mm thick
- Immediate post menstrual phase -1-2mm thick
- Proliferative phase - 2- 4mm thick
- Mid secretory phase - 7-8mm thick

There is some reduction of 5-6mm in the thickness of endometrium in the immediate premenstrual phase.

PHASES OF MENSTRUAL CYCLE:

The normal menstrual cycle is divided into two main phases.

1. The proliferative phase.
2. The secretory phase.

Estrogen predominates in the proliferative phase, and the progesterone action predominates in the secretory phase.

1. THE PROLIFERATIVE PHASE:

This phase generally lasts two weeks but may fluctuate between one to twenty days. This phase is further subdivided into early, middle and late proliferative phases [9,12].

a) The early proliferative phase:

This phase occurs between fourth to seventh day of a twenty eight day cycle. The glands are sparse, narrow and straight with a low columunar epithelial lining. Their nuclei are small, oval and the chromatin dense. Nucleoli are inapparent. There is evidence of mitotic activity both in the glands and the stroma, and the stroma remains dense in appearance. As the effect of estrogen steadily increases, the endometrium gradually shifts to the mid proliferative phase.

b) Mid proliferative phase:

This phase occurs between eight to tenth day of a twenty eight day cycle and the characteristic change in this stage is increase in the height of the endometrium due to stromal edema induced by estrogen. The glands become tortuous and elongated. Their epithelial cells become tall columnar with large, oval nuclei and dense chromatin. Nucleoli are apparent and many cells show mitosis. The stroma is made up of spindle shaped cells with scanty cytoplasm and large fusiform nuclei, separated by interstitial edema.

c) Late proliferative phase:

This phase occurs between eleventh to fourteenth day of a twenty eight day cycle. The stromal edema subsides, the tortuosity of the glands are increased, and their lining epithelial cells show a pseudo stratified appearance. The nuclei are large with prominent nucleoli. At this time tiny granules of glycogen appear at the basal part of glandular cells. The granules stain red with Periodic acid Schiff stain. The stroma is compact with large and proliferated stromal cells with prominent nucleoli.

2. THE SECRETORY PHASE:

The normal secretory phase lasts approximately for fourteen days. Grossly, secretory endometrium is 3 to 5 mm thick and appears creamy yellow. At this phase, there is an overlap of both the proliferative and secretory activity, and the endometrial glands show both mitotic activity and secretory activity. This phase is divided into early secretory phase, mid secretory phase and late secretory phases [9,12].

a) Early secretory phase:

The appearance of the sub nuclear vacuolations is a characteristic feature of the early secretory phase. This phase lasts from the second postovulatory day to the fifth postovulatory day. The pseudo stratified appearance of the epithelium disappears and the glands become more tortuous.

b) Mid secretory phase:

This phase lasts from fifth day after ovulation till the eleventh day. The glands are irregular, in contrast to round or oval pattern seen in the proliferative phase. The luminal side of the glandular cells have secretory vacuoles. Apocrine secretions are seen in the cytoplasm in the luminal side of the cell. So the apical surface of the cells are rough and indistinct. The nuclei of epithelial cells are arranged in linear pattern and they are round and vesicular. Stromal edema begins on the seventh day after ovulation and peaks at nine and ten days after ovulation. On ninth day after ovulation, groups of spiral arterioles become prominent, and they grow thicker, larger, and spirally twisted.

Ten days after ovulation, the stromal edema starts to regress and stromal cells become decidualized. This change first appears as a solidification of the stromal cells around the spiral arterioles. The surrounding areas have a loose edematous pattern. Eleven days after ovulation, the stromal edema decreases and decidual change of the stromal cells is more pronounced. The regression of the stromal edema is the result of decreasing levels of estradiol and progesterone, which in turn are the result of lysis of the corpus luteum.

c) Late secretory phase:

This phase is characterized by compact stroma without edema. Decidual change and endometrial granulocytes are seen in the stroma of

superficial zone. Stroma in the spongy zone is undifferentiated. The glands have a characteristic 'saw toothed' appearance in the central zone, the spongy part of the endometrium. Epithelial cells are tall columnar with abundant secretions. The glands of stratum compactum are few in number and are lined by flattened cells. As the cycle ends, the stromal edema regresses completely and decidualization spreads throughout the endometrium.

THE MENSTRUAL PHASE:

If pregnancy has not occurred, the late secretory phase enters to the menstrual phase which starts 14 days after ovulation. This phase is characterized histologically by crumbling of the stroma, glandular collapse and haemorrhage. On the second day of menstruation, scattered stromal cells and remnants of glandular epithelium are found admixed with fresh blood and aggregates of neutrophils [9].

REGENERATION:

Regeneration is in progress before the cessation of the menstrual flow and is complete by the time bleeding stops. The regeneration process starts in the glands retained in the basal layer and part of functional layer which are retained [9].

DEFINITION AND CLASSIFICATION OF DUB

DUB is defined by various authors as follows:

Sutherland (1949) [13] defined DUB as all forms of abnormal uterine bleeding (AUB) for which no detectable pathology and physical signs can be detected by clinical examination. Author divided DUB into,

- Apparently normal endometrium
- Irregular shedding
- Irregular ripening of the endometrium
- Endometrial atrophy
- Endometrial hyperplasia

DUB is defined by **Vory and Neri (1967,1970)**[14,15] as AUB for which the cause cannot be detected by history taking, physical examination, pelvic examination, pap smear and uterine curettage. They observed that, DUB is caused not only by anovulatory states, but also corpus luteum insufficiency, shortened cycles and classified DUB based on clinical features and etiology.

TABLE 1: CLASSIFICATION OF DUB BASED ON CLINICAL FEATURES AND ETIOLOGY

Type	**Cycle**	**Type of bleeding**	**Cause**
I. Ovulatory a) Follicular Abnormality	a) Short proliferative phase	Short cycles, normal bleeding.	Hypersensitivity of ovary
	b) Long proliferative	Long cycle, normal bleeding	Slow development of follicle.
b)Corpus luteum Abnormality	a) Insufficiency	Premature spotting, short cycles	Irregular ripening of endometrium
	b) Prolonged	Prolonged cycle with excessive bleeding	Irregular shedding of the endometrium
II. Anovulatory	a) Cyclic	Normal cycle with excessive bleeding	High peak estrogen level. Proliferative Endometrium
	b) Acyclic	Irregular excessive bleeding	Continuous high levels of estrogen, Hyperplastic endometrium
		Irregular scanty bleeding	Continuous low levels of estrogen, Atrophic endometrium.

In 1981 Ackerman[16] stated DUB as bleeding not associated with an organic cause in women of child bearing age.

According to **Kurman Robert J** (1982)[17] DUB is the term used for bleeding that is not due to any underlying organic pathology and is therefore similar to AUB, resulting from derangements in the amount or duration of estrogen and progesterone effects on the endometrium.

According to **Telinde (1997**)[18] DUB includes any condition of abnormal uterine bleeding in the absence of infection, pregnancy, neoplasm or other intra uterine lesions. He also observed that such bleeding is often the result of hormonal dysfunction that inhibits ovulation .

Dutta in 2001[19] defined DUB as a state of abnormal uterine bleeding without any detectable organic pathology like inflammation, pregnancy or tumor.

According to **Jeffcoate (2002)**[20] all forms of abnormal uterine bleeding for which an organic pathology cannot be found, are grouped under DUB.

He classified DUB into,

- Anovulatory DUB which is due to absent corpus luteum.
- Ovulatory DUB causing polymenorrhoea and polymenorrhagia
- Corpus luteum defects.

Sherman Mark (2002)[21] stated that DUB is a diagnosis of exclusion in which the uterine bleeding is not associated with any organic pathology.

Shaws (2004)[22] defined DUB as menorrhagia without any disease or structural abnormality or diseases in the pelvis and with no other demonstrable extra genital cause for bleeding. He observed that the etiology is hormonal, due to increased levels of estrogen in the circulating blood which causes hyperplasia of the endometrium.

PERIMENOPAUSE:

In a study by Treloar, he observed that the average age for entry into the perimenopausal transition was 45.1, and the age range that included 95% of the women was 39–51years (Treloar AE, 1996). He also observed that the range of perimenopausal transition was 2 to 8 years, with a mean duration of 5 years[23].

WHO Scientific Group 1996 Research on the menopause states that "The term perimenopause include the period immediately before the menopause (when the biological, endocrinological, and clinical features of approaching menopause commence) and the first year after menopause[24].

Perimenopause is the period 2-8 year preceding menopause and one year after the final menses (WHO).However a better definition is, the phase preceding the onset of menopause, occurring around 40-50

years of age (beginning at age 47.5, lasting for 4 years) during which the regular cycle of a woman transitions to irregular cycles[2].

Joseph et al states that the term perimenopause, which literally means “about or around the menopause,” begins at the same time as the menopausal transition and ends one year after the final menstrual period[25].

Definition of Perimenopausal Bleeding:

Perimenopausal bleeding refers to the symptoms of prolonged, excessive, acyclic bleeding or unexpected bleeding , regardless of the diagnosis or cause[26].Almost 90% of women will have four to eight years of menstrual cycle irregularities before menopause. It is important to differentiate irregular bleeding during the perimenopause from abnormal uterine bleeding (AUB). AUB is more common in the perimenopausal years. AUB refers to the symptoms of prolonged,excessive, acyclic bleeding or unexpected bleeding , regardless of the diagnosis or cause, whereas dysfunctional uterine bleeding (DUB) is a term which refers to any abnormal bleeding from an essentially normal uterus[26].

During the perimenopause, AUB is related to both altered hormonal function of the ovaries and to uterine abnormalities.The perimenopause is characterized by increasing unpredictability and irregularity of menstrual cycles[27]. Physiologic changes include an increasing incidence of short and long follicular phases, anovulation,

defective ovulation, corpus luteum insufficiency and erratic cycles. Most often these changes are associated with premenstrual follicle stimulating hormone (FSH) increase. Throughout the perimenopausal transition, there is a significant increase in the incidence of DUB due to anovulation[28].

Though uncommon in the perimenopausal age, the rate of endometrial neoplasia begins to increase sharply at age 40-50[29]. Perimenopausal bleeding may be a sign of atypical hyperplasia of endometrium, which if undiagnosed and untreated may progress to endometrial carcinoma[28]. Although changes in bleeding pattern in perimenopausal patients are normal, it is critical for clinicians to recognize abnormal bleeding patterns so that proper investigations can be carried out.

Causes of AUB in Perimenopausal Women[26]

TABLE 2: DIFFERENTIAL DIAGNOSIS OF ABNORMAL UTERINE BLEEDING IN PERIMENOPAUSAL WOMEN

A	**Organic causes**
	Benign reproductive tract diseases Leiomyomata uteri Polyps Adenomyosis Endometritis Cervicitis/Vaginitis
	Premalignant/Malignant pelvic lesions Endometrial hyperplasia Endometrial adenocarcinoma
B	**Systemic diseases** Coagulation disorders Hypothyroidism Liver disease
C	**latrogenic causes** Hormone therapy Contraceptive devices/Hormones Anticoagulation therapy
D	**Anovulation – Dysfunctional Uterine Bleeding**

In the perimenopausal age, AUB is frequently related to DUB, which is either ovulatory or anovulatory. Defects in local endometrial hemostasis leads to ovulatory DUB,while systemic disorders that occurs due to the imbalance of sex steroids in the absence of anatomic lesions leads to anovulatory DUB .

Alternatively, abnormal bleeding can occur secondary to organic etiologies within the uterus that affect endometrial hemostasis, such as leiomyomas,polyps,endometrial hyperplasia and neoplasia. Coagulopathies though uncommon, should be considered in the differential diagnosis of AUB. Uterine leiomyomas are common, especially in women in the fourth and fifth decades of life[28].

ANOVULATORY DISTURBANCES:

Endometrial atrophy (Insufficient follicular development):

This is characterized by a complete lack of endometrial response to ovarian hormones. Histopathologically epithelial and stromal cells are small, with very sparse glands lined by low cuboidal epithelium .The nuclei are small, round with dense chromatin. The cytoplasm is scanty with no mitoses. The stroma is made of densely packed, spindle cells[9, 27].

Deficient proliferation:[9]

Due to central hypogonadotrophic or ovarian damage, if a growing follicle does not reach maturity, it remains functionally inadequate and

little estrogen will be produced with two consequences.

1. In the early follicular phase , diminished concentration of FSH causes insufficient feedback stimulation and the LH levels will remain low. As a result,the LH peak will not develop and ovulation does not take place.
2. Due to understimulation, the endometrium will not proliferate adequately. The LH concentration is too low for the induction of ovulation, but it causes sporadic luteinization in the insufficient follicle.

Histologically, the endometrial glands and stroma show retardation of growth. The glands are narrow and straight with low columnar epithelium and has densely arranged chromatin rich nuclei in single row with scanty cytoplasm. The estrogen receptor content is low. The stromal cells are spindle shaped, small, poorly differentiated and are densely packed. The height of the endometrium is moderate, with slightly irregular surface. Mitoses are rare.

Irregular proliferation: (persistent ovarian follicle): [9,10]

Although a follicle has matured normally, ovulation may not take place because of a central defect in LH stimulation, or of ovarian damage or hyperstimulation with FSH. The follicle which is unruptured and persistent, produce estrogen beyond the proliferation phase for a number of days and regress slowly, resulting in anovulatory shedding which

occurs at the same time as menstrual shedding, or it may be more or less delayed,depending upon how long the follicle persists.

Histologically the growth of the glands and stroma exceeds that of the normal proliferative phase. The glands are lined by a pseudostratified or stratified high columnar epithelium,with varied distribution which is either closely packed or widely dispersed. On immunohistochemical analysis, the nuclei of the proliferating glandular epithelial cells show strong staining for estrogen receptors. The stroma is irregularly edematous and composed of densely arranged spindle cells. The spiral arterioles are underdeveloped,and thin walled venules are found.

At times, irregular proliferation may develop into atypical hyperplasia, without entering the stage of simple glandular hyperplasia. Perimenopausal bleeding patients with irregular proliferation should be carefully followed up and their estrogen levels analyzed, to ensure that a long standing unopposed estrogen stimulation of the endometrium is not overlooked.

Irregular proliferation may develop focally, and these areas show delayed shedding or no shedding with menstruation, because their reticulum fibres are not dissolved .This can give rise to polyps which may grow and become pedunculated.

OVULATORY DISTURBANCES: [9,30]

The Corpus luteum insufficiency- deficient secretory phase:

This comprises many disturbances in corpus luteum function of central or ovarian origin with or without preceding abnormal development of follicle. When the hormonal balance shifts in favour of estrogen,the stimulatory effect of progesterone on the endometrium become deficient.

Irregular shedding (Persistent corpus luteum):[8]

Failure of regression of a normally developed corpus luteum leads to continuous secretion of progesterone and the menstrual bleeding will be delayed and prolonged. Such a persistence of the corpus luteum may be caused by hyperstimulation from chorionic gonadotrophin or pituitary as in intrauterine or extrauterine pregnancy.

Irregular shedding is characterized by admixture of endometrial fragments in various stages of regression and dissociation.So the glandular lumen become star shaped.The cytoplasm of most of the glandular cells is clear and contain abundant glycogen.Nuclei are shrunken with dense chromatin.

Disordered Proliferative Endometrium:[9]

In disordered proliferative endometrium there is absence of pattern uniformity, due to dyssynchronous growth of the functional layer of endometrium. In some areas, the glands are narrow, tubular,and with

abundant stroma, while in other areas the glands are cystically dilated with varying degree of shallow budding. The ratio of gland to stroma is 1:1. Thus, disordered proliferation,which is commonly observed in perimenopausal women, differs from simple hyperplasia without cytological atypia(gland to stroma ratio is 3:1) by its relatively normal gland to stromal ratio.

Endometrial Hyperplasia:

Endometrial hyperplasia is a noninvasive proliferation of the endometrium resulting in histomorphologic pattern of endometrial glands with irregular shapes and varying size[31].Endometrial exposure to prolonged levels of unopposed estrogen, that commonly occurs with anovulation in the perimenopausal age may result in the development of endometrial hyperplasia[32].Endometrial hyperplasia comprises of both epithelial and stromal proliferations that has varied morphological patterns.

Endometrial hyperplasia may be the consequence of either,

1. A persistent follicle with high levels of estrogen for a long period.
2. Repeated follicular atresia with hyperplasia of theca cells that secrete estrogen.
3. Repeated anovulatory cycles or polycystic ovarian disease.
4. Recurrent severe luteal defect.

Other causes are exogenous estrogen administration and

endogenous conditions that produce excessive estrogen like hilar cell hyperplasia, stromal hyperplasia, granulosa cell tumours and thecomas.

Harold Fox in 1984[33] recognized four forms of hyperplasia,

1. Cystic glandular hyperplasia
2. Adenomatoid hyperplasia
3. Glandular hyperplasia with architectural atypia
4. Glandular hyperplasia with cytologic atypia.

Kurman and Norris in 1986[34] classified hyperplasias into simple and complex as follows:

• Hyperplasia	:	Simple
		Complex
• Atypical hyperplasia	:	Simple
		Complex

M.C. Anderson in 1991[35] classified endometrial hyperplasia into,

1. Cystic hyperplasia-graded as

- Mild
- Moderate
- Severe

2. Atypical hyperplasia

a) Architectural atypia

b) Cytological atypia, which is further graded into

- Mild

- Moderate
- Severe

Ronnett B.M and Robert J. Kurman in 2002[30] defined endometrial hyperplasia as a lesion with increase in the gland to stroma ratio with predominant proliferation of irregular sized and shaped glands. He classified hyperplasia in to

1. Hyperplasia without cytologic atypia (non atypical hyperplasia)
    a) Simple
    b) Complex
2. Hyperplasia with cytologic atypia (atypical hyperplasia)
    a) Simple
    b) Complex

Simple or complex hyperplasias were classified on the degree of glandular crowding.

Current classification of endometrial hyperplasia accepted by both the International society of Gynaecological Pathologist (ISGP) and WHO (2003)[36] divides the hyperplasia on the basis of architectural features in to simple and complex, and on the basis of cytological features into typical or atypical.[37]

TABLE 3 :WORLD HEALTH ORGANIZATION CLASSIFICATION OF ENDOMETRIAL HYPERPLASIA:[36,38]

Typical Hyperplasias	Atypical hyperplasias
1. Simple hyperplasia without atypia	1.Simple atypical hyperplasia
2.Complex hyperplasia without atypia(syn.adenomatous hyperplasia without atypia)	2.Complex atypical hyperplasia (syn.adenomatous hyperplasia with atypia)

TYPICAL HYPERPLASIA:

Typical hyperplasia comprises of :

- Simple hyperplasia without atypia.
- Complex hyperplasia without atypia

SIMPLE HYPERPLASIA WITHOUT ATYPIA:[39]

In simple hyperplasia without atypia, the endometrium is edematous and glassy. There are marked proliferative changes and increased mitotic activity of both the glands and stroma. The glands are cystically dilated and tortuous and have the characteristic "Swiss Cheese" pattern . The cells are tall and may be high cuboidal, columnar or pseudostratified, depending upon the degree of hyperplasia. The nuclei are elongated with dense chromatin and large nucleoli. The activity of alkaline phosphatase increases, which is directly proportional to the level of estrogen. Droplets of glycogen can be demonstrated.

The stromal cells are densely packed and plump spindle shaped with dense nuclei and scant cytoplasm. Granulocytes are absent. Cytologic atypia is rare. The reticulum fibres are irregularly distributed eventhough increased in number and thickness . The spiral arteries and arterioles are poorly developed and has a straight course. The amount and duration of hyperestrogenism determines the fate of simple hyperplasia.Regressive changes may appear if the level of oestrogen falls, but if hyperestrogenism persists simple hyperplasia progresses to complex hyperplasia.

COMPLEX HYPERPLASIA WITHOUT ATYPIA (ADENOMATOUS HYPERPLASIA WITHOUT ATYPIA):[40]

In complex hyperplasia without atypia, budding of the glandular epithelium occurs , and new glands are formed. The glands are lined by tall columnar, stratified epithelium from which epithelial papillae develop and protrude into the lumen. The nuclei are elongated, large, and rich in chromatin but basal polarity is maintained. The cytoplasm is scant with numerous mitoses. As the glands proliferate, the intervening stroma is compressed and gradually decreased, so that the glands are arranged in back to back position.

ATYPICAL HYPERPLASIA:[40]

The main feature which differentiates atypical hyperplasia from complex hyperplasia without atypia is the atypical cytology of the

glandular lining epithelium characterized by loss of axial polarity, nuclear pleomorphism, irregularity in the nuclear membranes, prominent nucleoli and dense chromatin.

Atypical hyperplasia comprises of:

- Simple atypical hyperplasia
- Complex atypical hyperplasia

SIMPLE ATYPICAL HYPERPLASIA :

Simple atypical hyperplasia is characterized by atypical glandular morphology superimposed on the architecture of simple hyperplasia without atypia. This pattern is extremely unusual.

COMPLEX ATYPICAL HYPERPLASIA (ADENOMATOUS HYPERPLASIA WITH ATYPIA):

Complex atypical hyperplasia is characterized by increased complexity of the glandular epithelium with irregular outgrowths and cytological atypia. Focal areas of nonendometrioid differentiation such as squamous morules may be present . The interglandular stroma is present but diminished due to the expansion and crowding of glands. Characteristic features of adenocarcinoma of the endometrium are absent.The assessment of cytological atypia is the key problem in assigning individual cases to one of the four different WHO categories of hyperplasia.

ENDOMETRIAL CARCINOMA:[40]

Endometrial carcinoma is a primary malignant epithelial tumour, arising in the endometrium.This malignant tumor shows a glandular differentiation and can invade into the myometrium with distant metastatic spread.

Though rare in perimenopausal age, the progression rate of untreated endometrial hyperplasia to carcinoma is significantly higher. Two types of tumors are identified

- Type I
- Type II

"Estrogen dependent tumours Type I" are low grade and associated with endometrial hyperplasia, such as atypical hyperplasia. Unopposed estrogen stimulation is the driving force behind type I group of tumours. This type is common in perimenopausal age group[41,42].

The second type **(**Type II) of endometrial carcinoma is less related to sustained estrogen stimulation and this type commonly occurs in postmenopausal age group [41,42] .

Endometriod adenocarcinoma is a primary endometrial adenocarcinoma with glands resembling the normal endometrium.Histopathologically, most common type of carcinoma of endometrium is the endometrioid adenocarcinoma,which shows glandular or villoglandular architecture and are lined by either simple

columnar or pseudostratified columnar cells . The differentiating feature of well differentiated endometrioid adenocarcinoma from atypical hyperplasia is by stromal disappearance between adjacent glands, i.e. cribriform, villoglandular patterns.

Both typical hyperplasias and atypical hyperplasias may regress spontaneously over months or years. However, atypical hyperplasia is a precancerous condition that may progress to malignancy and is best treated by hysterectomy. Hyperplasia without atypia is known to regress spontaneously after D&C or progestin treatment[42].Progression rate of untreated endometrial hyperplasia to carcinoma[28,43] is shown in table 4.

TABLE 4: PROGRESSION RATE OF UNTREATED ENDOMETRIAL HYPERPLASIA TO CARCINOMA

Type of hyperplasia	Progression to carcinoma
Simple hyperplasia without atypia	1%
Complex hyperplasia without atypia	3%
Simple hyperplasia with atypia	8%
Complex atypical hyperplasia	29%

The most common lesion that predisposes to endometrial adenocarcinoma is atypical endometrial hyperplasia. If left untreated, approximately 8% of patients with simple atypical hyperplasia and 29% of patients with complex atypical hyperplasia will progress to carcinoma[28].

In patients with atypia, if conserving the uterus is considered, a trial of hormonal treatment may be given. Steroid hormone receptor analysis plays an important role or may be an indication in this group of patients to predict their response to hormonal therapy. Steroid hormone receptor analysis also plays a role in predicting the prognosis of patients with these lesions.

ESTROGEN AND PROGESTERONE RECEPTORS: [44,45]

The estrogen receptor (ER) and progesterone receptor (PR) , members of the steroid hormone receptor family, act as hormone dependent activators of transcription. Two estrogen receptors are identified, ERα and ERβ(**Fig.3**). ERα (60-66 kD protein) and ERβ(51-61kDa protein) located at chromosome 6 and chromosome 14 respectively and are translated from two different genes.

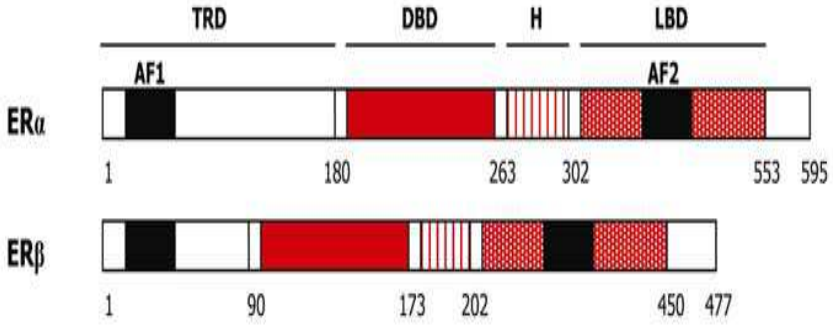


Figure 3: Structure of estrogen receptor (ERα and ERβ)

ER alpha and beta are expressed in tissues of different types, but their expression pattern varies. The ERα is found in endometrium[46], stromal cells of ovary, neoplastic cells of breast and in the hypothalamus.

The ERβ protein is found in kidney, lungs, intestinal mucosa, prostate, brain, heart, bone and endothelial cells[44,47].

The ratio of the concentration of ERα and ERβ subtype plays a major role in certain diseases[48,49]. The ability of the estrogen receptor modulators to promote ER interactions with various proteins is the mechanism for the concept of selective estrogen receptor modulators(SERM).These proteins may be transcriptional co activator or co repressors[50]. The ratio of the co activator to the co repressor protein varies in different tissues [51] .

The progesterone receptor (PR) NR3C3 is a nuclear receptor subfamily 3, group C, member 3,and it is an intracellular steroid receptor that specifically binds progesterone. PR exists as two isotypes, A and B with molecular weights of approximately 95 kD protein and 120 kD protein(**Fig.4**). PRA and PRB are both identical in their sequence, but PRA lacks 164 amino acids at the N-terminus, which makes it the shorter of the two proteins [52,53] **.**

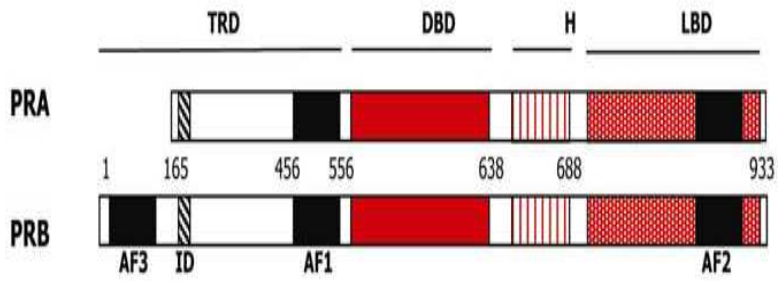


Figure 4: Structure of progesterone receptor (PRA and PRB)

Many functional domains can be distinguished in both ERs and PRs. They are the transcription regulating domain (TRD), hinge region (H), DNA binding domain (DBD), ligand binding domain (LBD), activation function domain (AF1-3) and an inhibitory domain (ID).Numbers indicate the location of amino acid(Fig.3&Fig.4).

Estrogen is needed for the induction of the progesterone receptors. Progesterone has different tissue specific effects in humans,[54] and is a regulator of normal female reproductive function. Estrogen amplifies the action of progesterone. The action of progesterone depends on the presence of progesterone receptor. The expression of the progesterone receptors are up regulated by estrogen through the estrogen receptors [55,56].

MODE OF ACTION OF ESTROGEN AND PROGESTERONE RECEPTORS:

The ER ,PR receptors are associated with heat shock proteins in the absence of ligand. After binding the ligand , the conformation of the receptor changes and dissociation of heat shock proteins occur. They then form receptor hormone complex dimers, homodimers for example ERα-ERα and heterodimers for example ERα-ERβ. Both ERα and ERβ are located in the nucleus of the cell in the absence of ligand,and PR is present either in the cytoplasm or the nucleus, in unliganded state.

After binding of ligand,the receptor gets activated through phosphorylation and both receptors ER and PR predominantly localize to

the nucleus. In the nucleus, these dimers, homo or heterodimers bind to specific hormone response elements (ERE or PRE) on the DNA. After the receptor dimer binds to the DNA, many coactivators, corepressors and transcription factors bind, after which the target genes are transcribed[57].(**Fig.5**)

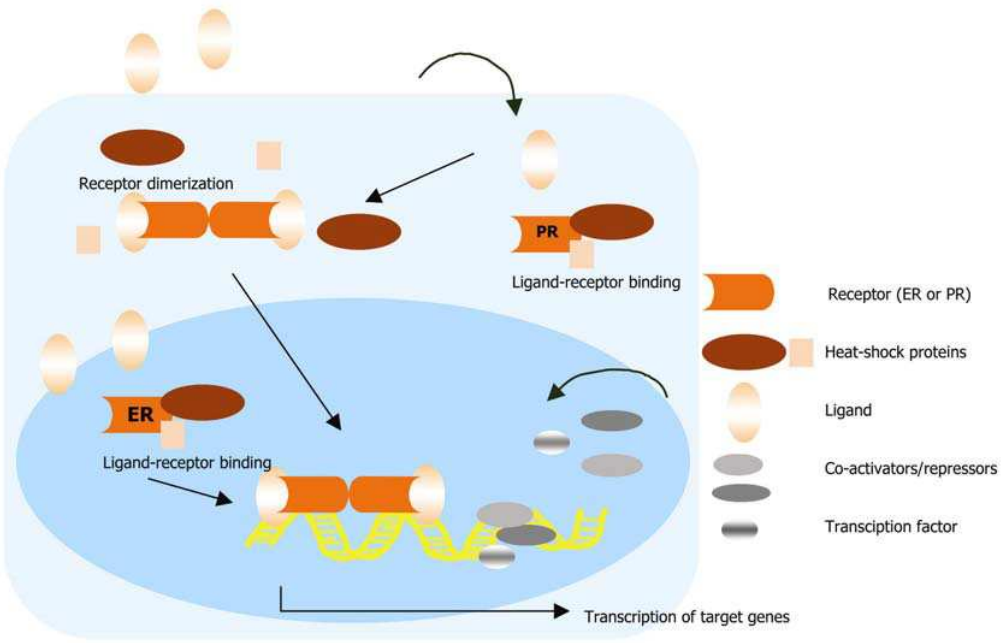


Figure 5: Mode of action of estrogen and progesterone receptors.

ER AND PR IN NORMAL ENDOMETRIUM:

The endometrium expresses estrogen (ER) and progesterone receptors (PR). Throughout the menstrual cycle, the concentrations of both estrogen receptor (ER) and progesterone receptor (PR) undergo considerable variations in the human uterus,in response to changing hormonal levels , and a variable expression of the steroid receptors in the cells of epithelium and stroma of the endometrium occurs.

The differential expression of steroid receptors ERα, ERβ, PRA and PRB in normal and atrophic endometrium were observed by Mylonas I, Snijder and many authors[58,59].In glandular and stromal cells of the endometrium, the basalis and functionalis, significant changes were noted in the receptors during the menstrual cycle.

In the late proliferative phase of the menstrual cycle,the estrogen receptor expression reached a maximum, in the endometrium. The expression of estrogen[48] receptor in the glandular epithelial cells declined more gradually [58] during the early secretory phase of the cycle whereas, it declined sharply in the stromal cells and smooth muscle cells.During mid secretory and late secretory phases, an increase in expression of estrogen receptor was observed in the predecidualizing cells of the stroma and smooth muscle cells[58].

The number of progesterone receptors in the glandular epithelial cells of the endometrium changed significantly but not in the stromal cells and smooth muscle cells. In the early secretory phase,the progesterone receptor expression in the endometrial glands reached a maximum and then decreased suddenly. During mid secretory phase and late secretory phases, PR immunostaining was moderate in the stromal cells in contrast to the glandular epithelium which showed very weak staining or no staining [58].

ER AND PR IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM:

Proliferative disorders of the endometrium are caused due to the autocrine and paracrine actions of estrogen and progesterone in the epithelial and stromal cells. There is a progressive loss of PR expression in stromal cells of endometrium and this may induce abnormal proliferation of endometrium due to disrupted hormonal balance[60].

Endometrial carcinoma being one of the commonest gynecologic malignancies, various prognostic factors have been studied to improve the treatment and follow up. Prognostic significance of the steroid hormone receptors has been well established in breast carcinoma. Several studies on breast carcinoma have confirmed the validity of ER,PR immunohistochemistry and has shown their prognostic and therapeutic usefulness. Analogous studies in endometrial carcinoma are few and limited to clinocopathological correlation.

Patients with significant numbers of both estrogen and progesterone receptors in advanced endometrial carcinoma tend to have an indolent clinical behavior than patients with absent or low level of these receptors.The response rate to progestins for the lesions which are progesterone receptor rich are better compared to progesterone receptor poor lesions [56].

Many studies have observed the effects of the steroid receptor status on survival of the patient. In a study by Geisinger et al, on the survival status, he found that quantitative levels of the progesterone receptor was significantly related to survival. Creasman et al. observed that estrogen receptor positive status, progesterone receptor positive status, and combined estrogen receptor and progesterone receptor positive status had a significantly greater disease free survival than those with estrogen receptor and progesterone receptor negative tumors. Myolonas. I, observed that the loss of receptor positivity for ERα in patients with endometrial adenocarcinoma resulted in a poorer survival, while the loss of receptor positivity for ERβ did not affect the survival[61,62,63].

IMMUNOHISTOCHEMISTRY :

Immunohistochemistry has become necessary and an interesting part of diagnostic pathology due to the accuracy and ease that it imparts to the studies of tissues at the cellular level. Hence, it is becoming widely used in diagnostic research procedures.

SCORING SYSTEM FOR ER AND PR IHC STAINING:

Estrogen and progesterone receptors show nuclear positivity. They are scored by the proportion of tumor cells showing positivity and the intensity of the reaction. Both the scores are summated to give a total score. Different scoring systems used to score estrogen and progesterone receptors include H scoring system,[64] Quick scoring system[65]and Allred

scoring system[66] .Quick scoring system that was proposed by Barnes et al is widely used worldwide as the score correlates well with the chance of patient response to hormonal therapy [66].

The current study has been undertaken to evaluate the histomorphological profile of endometrium in perimenopausal bleeding and to evaluate the steroid hormone receptor status in endometrial hyperplasias using immunohistochemical method.

MATERIALS AND METHODS

This study is a prospective study of the histomorphological profile of endometrium in perimenopausal bleeding conducted in the Department of Pathology, Government Tirunelveli Medical College and Hospital during the period of June 2010- October 2012.

SOURCE OF DATA:

The endometrial curettage specimens received in the Department of Pathology , Government Tirunelveli Medical College and Hospital during the period of June 2010- October 2012,that are clinically diagnosed DUB/AUB patients in perimenopausal age group of 40-50 years.

SAMPLE SIZE:

200 cases.

INCLUSION CRITERIA:

All cases that are clinically diagnosed as DUB/AUB and are in the perimenopausal age group of 40-50 years.

EXCLUSION CRITERIA:

Endometrial curettage from patients of other age groups and postmenopausal bleeding cases.

METHOD OF COLLECTION OF DATA:

The endometrial curettage specimen was collected from 200 clinically diagnosed DUB/AUB patients in perimenopausal age group of 40-50 years.The endometrial curettage samples were fixed in 10% formalin, histopathological slides were prepared and stained with Hematoxylin and Eosin stain(**Appendix 1**). All cases of hyperplasia including simple hyperplasia without atypia,complex hyperplasia without atypia,complex atypical hyperplasia and carcinoma were selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for ER and PR status. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemical analysis of estrogen and progesterone receptors were done in paraffin embedded tissue samples for all cases which were diagnosed as hyperplasias, including simple hyperplasia without atypia, complex hyperplasia without atypia, complex atypical hyperplasia and carcinoma using supersensitive polymer HRP system based on non biotin polymeric technology. 4 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done. The antigen is bound with mouse monoclonal antibody (Biogenex) against estrogen and progesterone receptors. The step by step procedure of immunohistochemistry is given in **Appendix -2.**

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analysed for the presence of reaction, cellular localization. The percentage of cells stained per 1000 cells counted on 40X power field and the intensity of reaction were analysed. Immunohistochemical scoring for ER and PR receptors were done with Quick score[65].

QUICK SCORING SYSTEM FOR EVALUATION OF ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION:

TABLE 5: SCORE FOR PROPORTION

Proportion of nuclei stained	**Score**
No nuclear staining	0
<1% nuclear staining	1
1-10% nuclear staining	2
11-33% nuclear staining	3
34-66% nuclear staining	4
67-100% nuclear staining	5

The proportion of the cells stained are observed and scored accordingly.

TABLE 6: SCORE FOR INTENSITY

Intensity of nuclear staining	**Score**
No staining	0
Weak staining	1
Moderate staining	2
Strong staining	3

The above two scores, score for proportion and the score for intensity(table 5 and table 6) are summated to a total maximum score of 8.Score of more than 2 is considered positive.

OBSERVATION AND RESULTS

TABLE 7: INCIDENCE OF DUB CASES COMPARED TO ALL GYNAECOLOGY CASES

S.No	Period	Total no. of Gynaecology Cases	No. of DUB Cases	Percentage
1	JUNE 2010-DEC 2010	1715	87	5.07%
2	JAN 2011-DEC 2011	2704	177	6.55%
3	JAN 2012-OCT 2012	2164	123	5.68%
	TOTAL	6583	387	5.88%

In the study period from June 2010 to October 2012, 6583 Gynaecology biopsy specimens were received in the Department of Pathology,Government Tirunelveli Medical college and Hospital. Among them, 387 cases were DUB cases. The average incidence of DUB cases were 5.88% as shown in Table 7 and Chart 1.

TABLE 8: INCIDENCE OF PERIMENOPAUSAL BLEEDING CASES COMPARED TO DUB CASES

Total DUB Cases	Perimenopausal Bleeding cases	Percentage
387	200	51.68%

Among the total number of DUB cases of 387, 200 cases were clinically diagnosed as DUB and are in the perimenopausal age group of 40-50 years, which constitutes about 51.68% as shown in Table 8 and Chart 2.

CHART 1

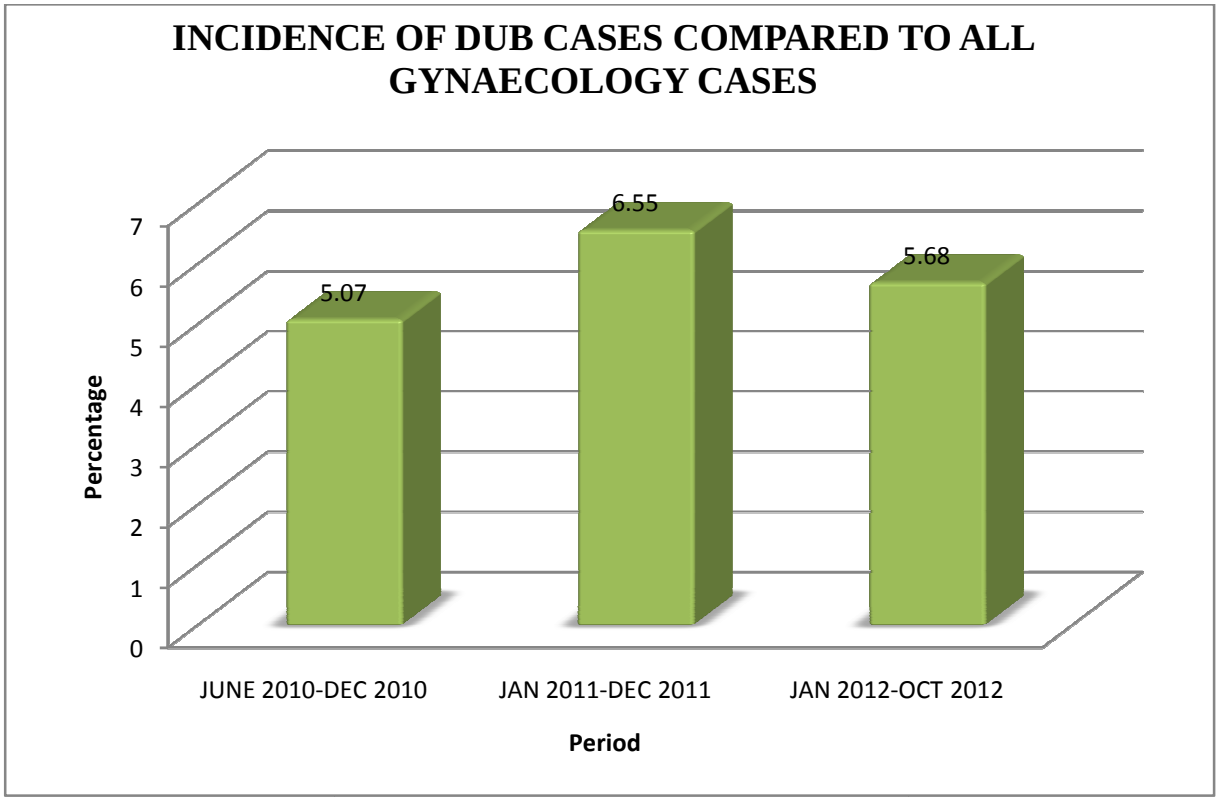


CHART 2

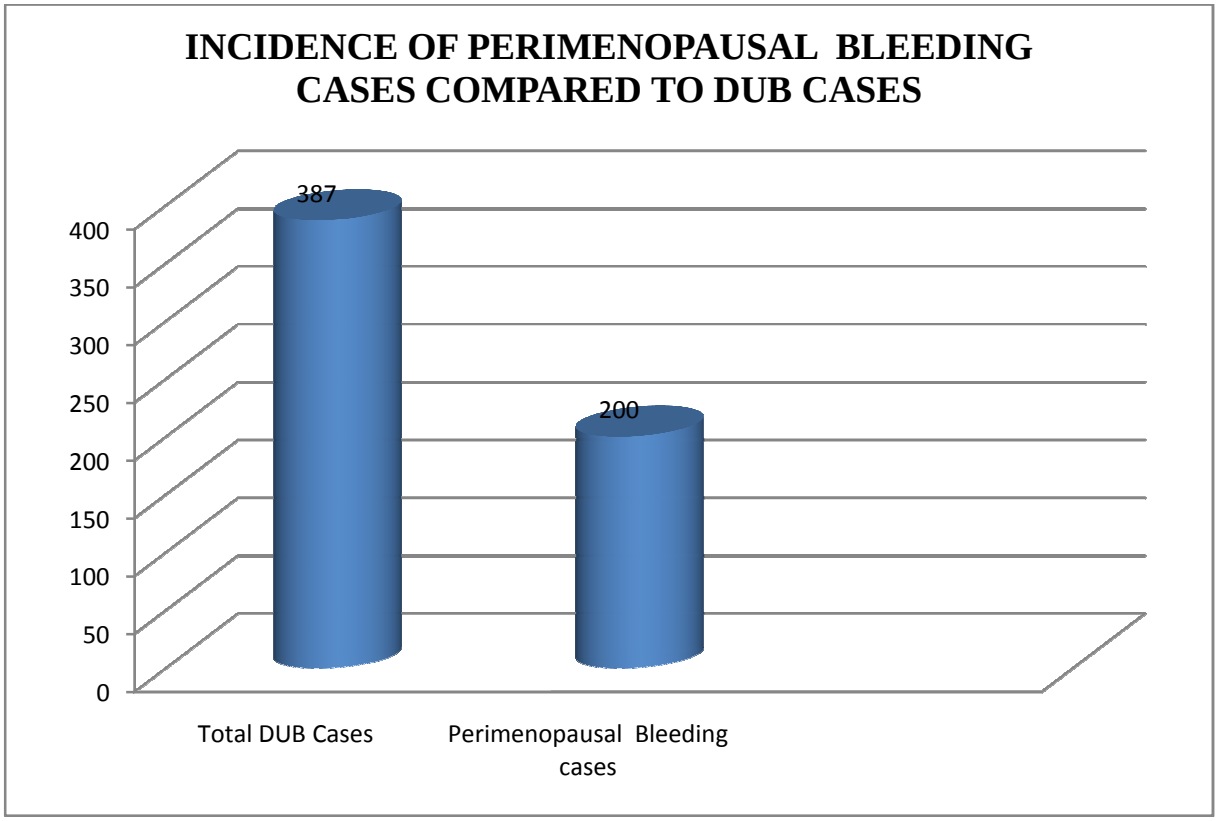


TABLE 9: AGE INCIDENCE OF PERIMENOPAUSAL BLEEDING CASES

Age group	No. of cases	Percentage
40-45 years	134	67%
46-50years	66	33%
Total	200	100%

Table 9 and Chart 3 depicts the age incidence of perimenopasual bleeding cases. Between the age group of 40-45 years, 134 cases (67%) were observed and between the age group of 46-50 years 66 cases (33%) were observed. So the maximum incidence of perimenopasual bleeding were seen in the age group of 40-45years (67%).

TABLE 10: DISTRIBUTION OF ENDOMETRIAL PATTERNS IN PERIMENOPAUSAL BLEEDING CASES

Type of Endometrium	No. of cases	Percentage
Proliferative endometrium and its variants	99	49.50%
Secretory endometrium and its variants	12	6%
Hyperplasias	48	24%
Others	41	20.50%
Total	200	100%

Among the 200 perimenopausal bleeding cases, proliferative endometrium and its variants constitute 99 cases (49.50%), secretory endometrium and its variants 12 cases (6%), hyperplasias 48 cases (24%), and other cases constitute 20.50% (41cases) (Table 10& Chart 4).

CHART 3

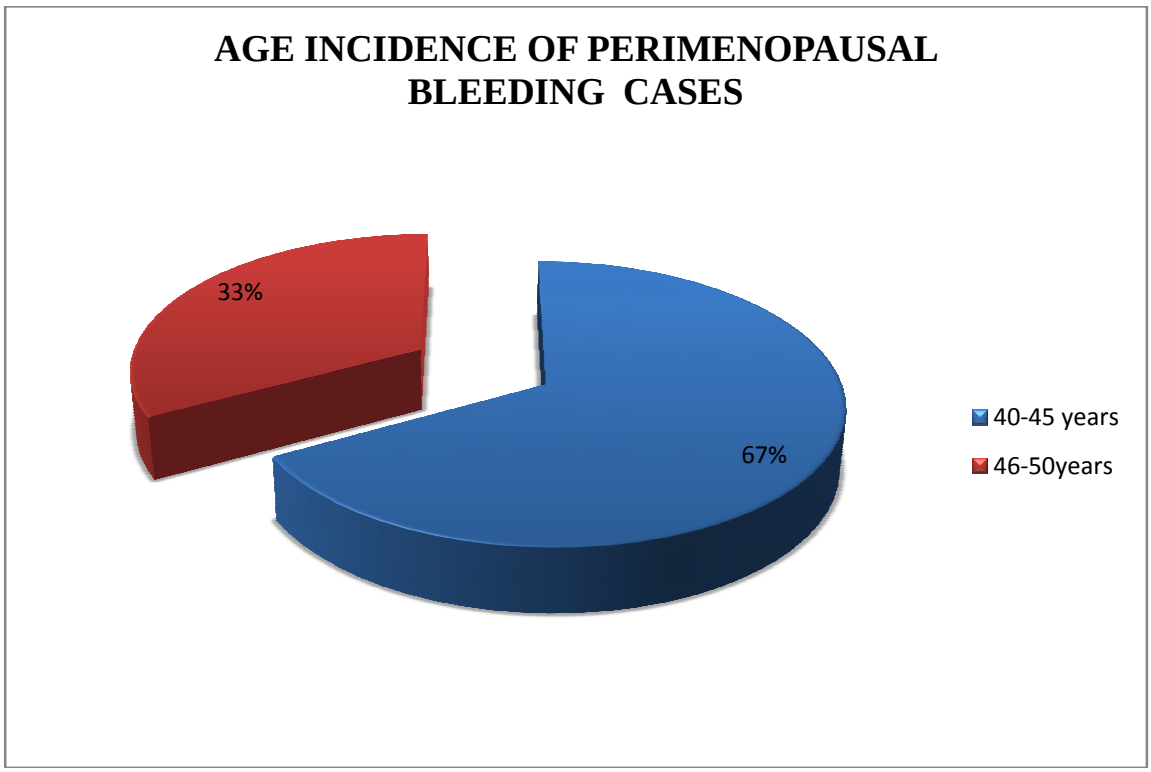


CHART 4

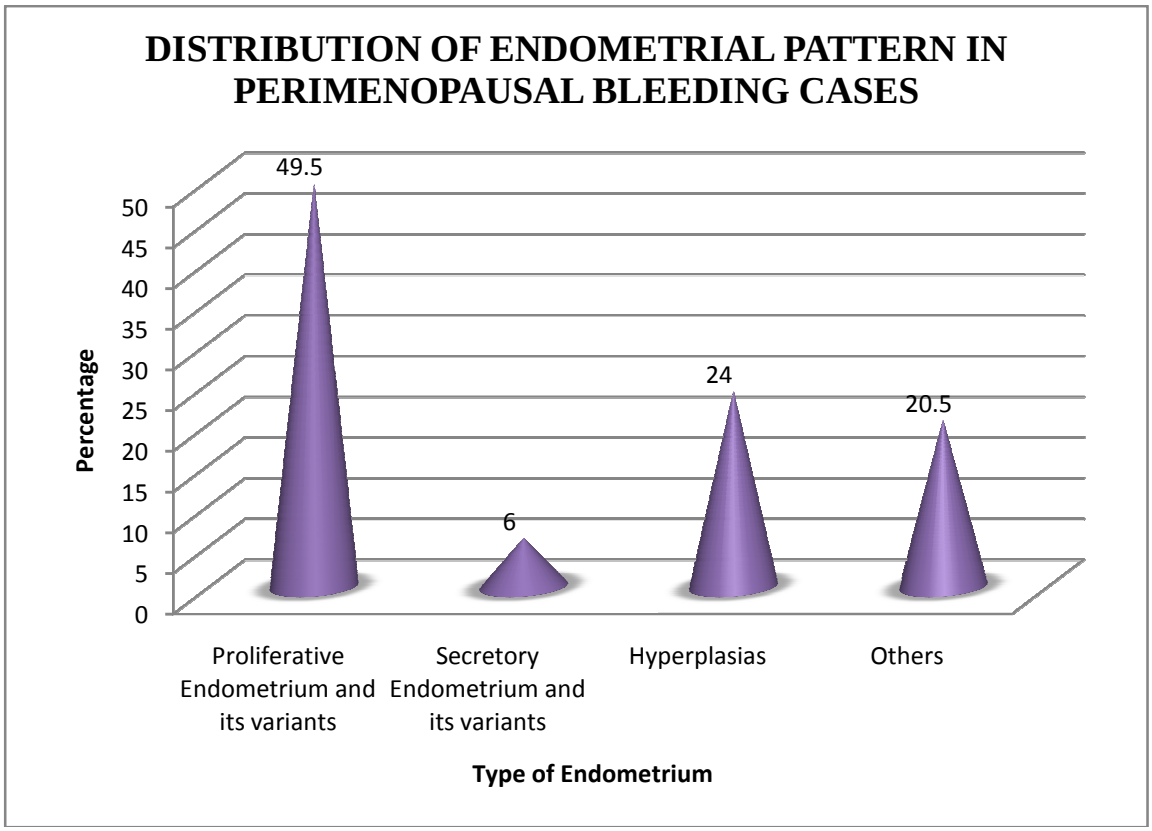


TABLE: 11 PROLIFERATIVE ENDOMETRIUM AND ITS VARIANTS

Type of Endometrium	No. of cases	Percentage
Proliferative endometrium	23	23.23%
Deficient proliferative endometrium	25	25.25%
Disordered proliferative endometrium	26	26.26%
Irregular proliferative endometrium	12	12.12%
Proliferative endometrium with cystic change	3	3.03%
Proliferative endometrium with disintegration	10	10.10%
Total	99	100%

In the total of 99 cases of proliferative endometrium and its variants, there were 23 cases (23.23%) of proliferative endometrium, 25 cases(25.25%)of deficient proliferative endometrium,26 cases(26.26%)of disordered proliferative endometrium, 12 cases(12.12%) of irregular proliferative endometrium, 3 cases (3.03%)of proliferative endometrium with cystic change, 10 cases(10.10%)of proliferative endometrium with disintegration (Table 11& Chart 5).

TABLE 12: AGE INCIDENCE OF PROLIFERATIVE ENDOMETRIUM AND ITS VARIANTS

Type of Endometrium	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Proliferative endometrium	21	91.3	2	8.7
Deficient Proliferative endometrium	14	56.0	11	44.0
Disordered Proliferative endometrium	11	42.3	15	57.7
Irregular Proliferative endometrium	8	66.7	4	33.3
Proliferative endometrium with cystic change	1	33.3	2	66.7
Proliferative endometrium with disintegration	8	80.0	2	20.0
Total	**63**		**36**	

Chi-square value =15.75, d.f.=5, P=0.008.

Between the age group of 40-45 years, there were 63 cases (63.63%), and in the age group of 46-50 years, 36 cases (36.36 %). Among the 23 cases with proliferative endometrium, 91.3% were in 40-45years age group and among the 10 cases of proliferative endometrium

with disintegration, 80% of them were in 40-45 years age group. Chi-square statistical test has been applied to find out if there is any association between the type of proliferative endometrium and age of the person. The significant p-value infers that disordered proliferative endometrium and proliferative endometrium with cystic change are more common among the women in the age group of 46-50 years compared to the women in the age group of 40 to 45 years. Similarly for the women in 40-45 years age group, proliferative endometrium and proliferative endometrium with disintegration is common(Table 12 & Chart 6).

CHART 5

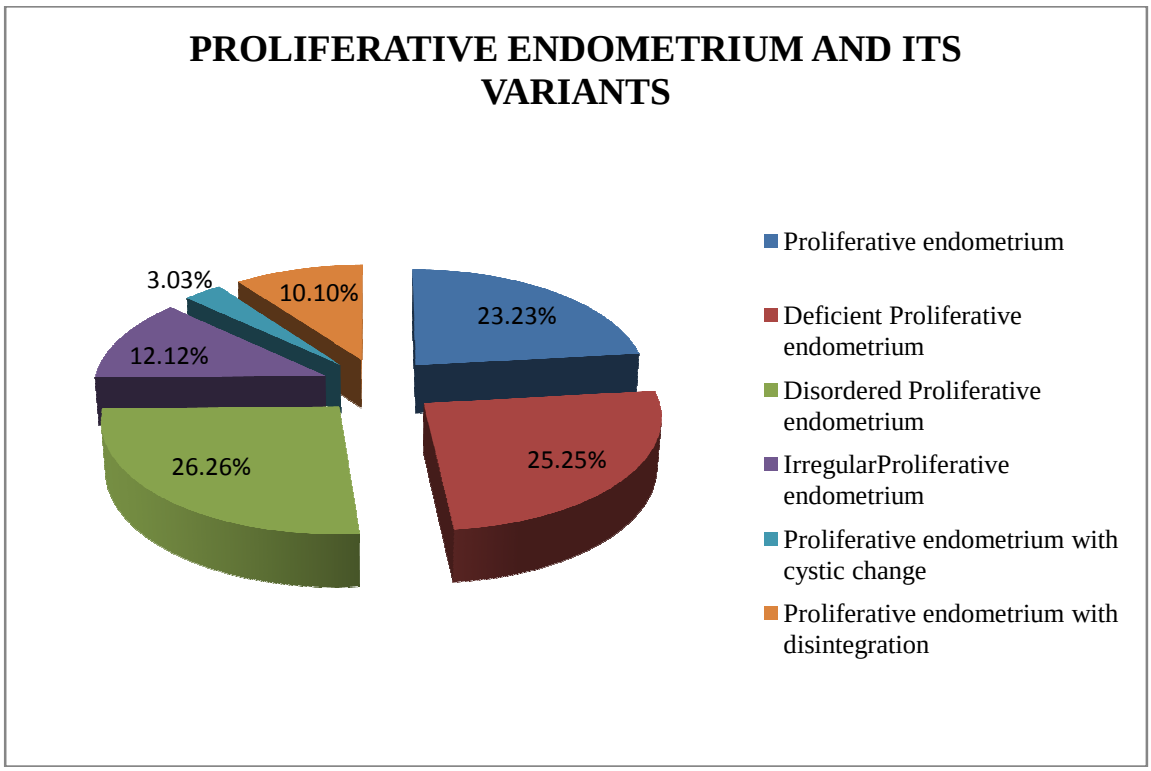


CHART 6

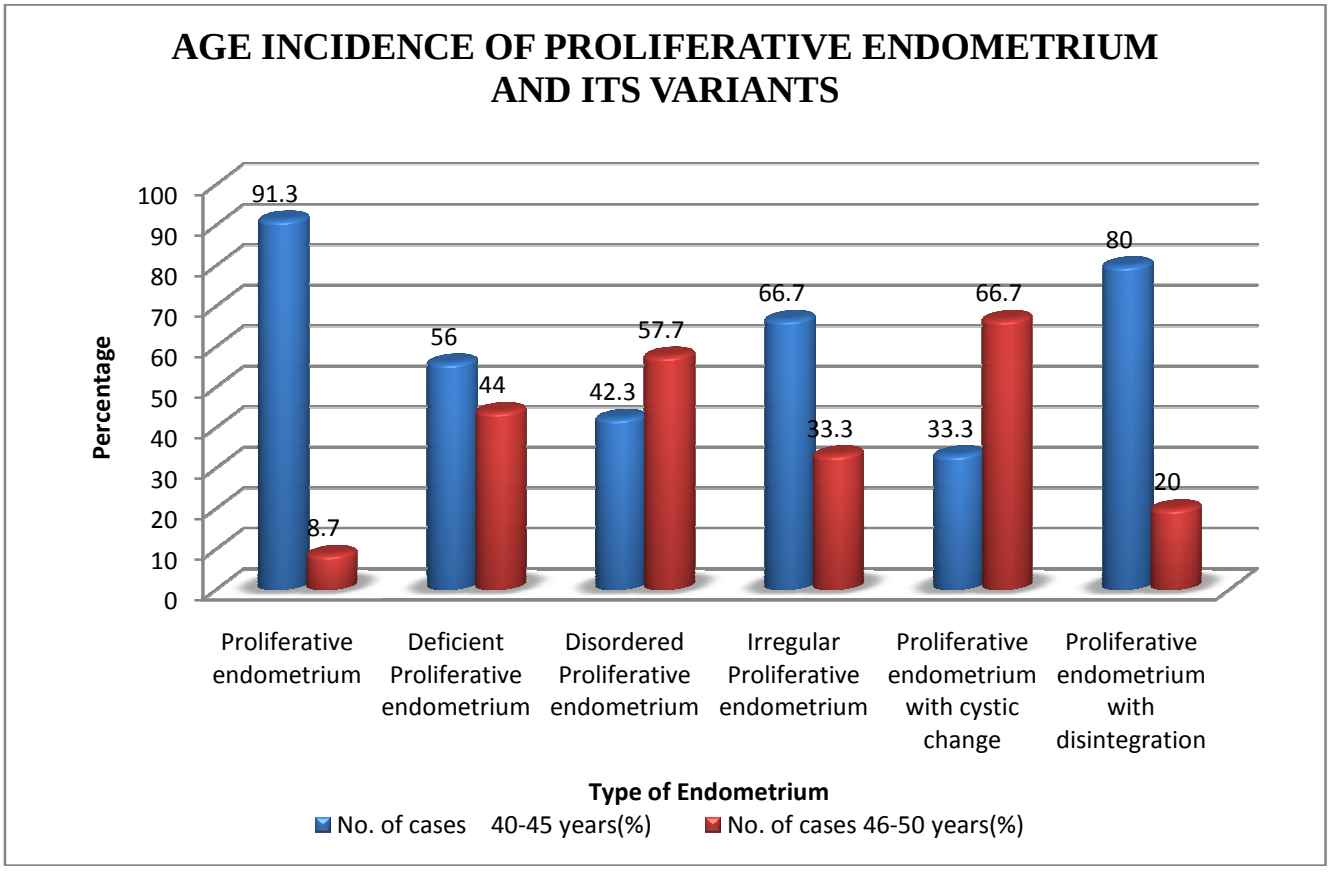


TABLE13: SECRETORY ENDOMETRIUM AND ITS VARIANTS

Type of Endometrium	No. of cases	Percentage
Early secretory endometrium	3	24.99%
Mid secretory endometrium	2	16.66%
Late secretory endometrium	1	8.33%
Deficient secretory endometrium	5	41.65%
Deficient secretory with stromal haemorrhage	1	8.33%
Total	12	100%

In the total of 12 cases of secretory endometrium and its variants, there were 3 cases (24.99%) of early secretory endometrium, 2 cases (16.66%) of mid secretory endometrium, 1 case (8.33%) of late secretory endometrium , 5 cases (41.65%) of deficient secretory endometrium and 1 case (8.33%) of deficient secretory endometrium with stromal haemorrhage(Table 13& Chart 7).

TABLE 14: AGE INCIDENCE OF SECRETORY ENDOMETRIUM AND ITS VARIANTS

Type of Endometrium	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Early secretory endometrium	3	100%	0	0%
Mid secretory endometrium	1	50%	1	50%
Late secretory endometrium	1	100%	0	0%
Deficient secretory endometrium	4	80%	1	20%
Deficient secretory with stromal haemorrhage	0	0%	1	100%
Total No.of cases	9		3	

Between the age group of 40-45 years, there were 9 cases of secretory endometrium, and in the age group of 46-50 years, 3 cases were observed(Table 14& Chart 8).

CHART 7

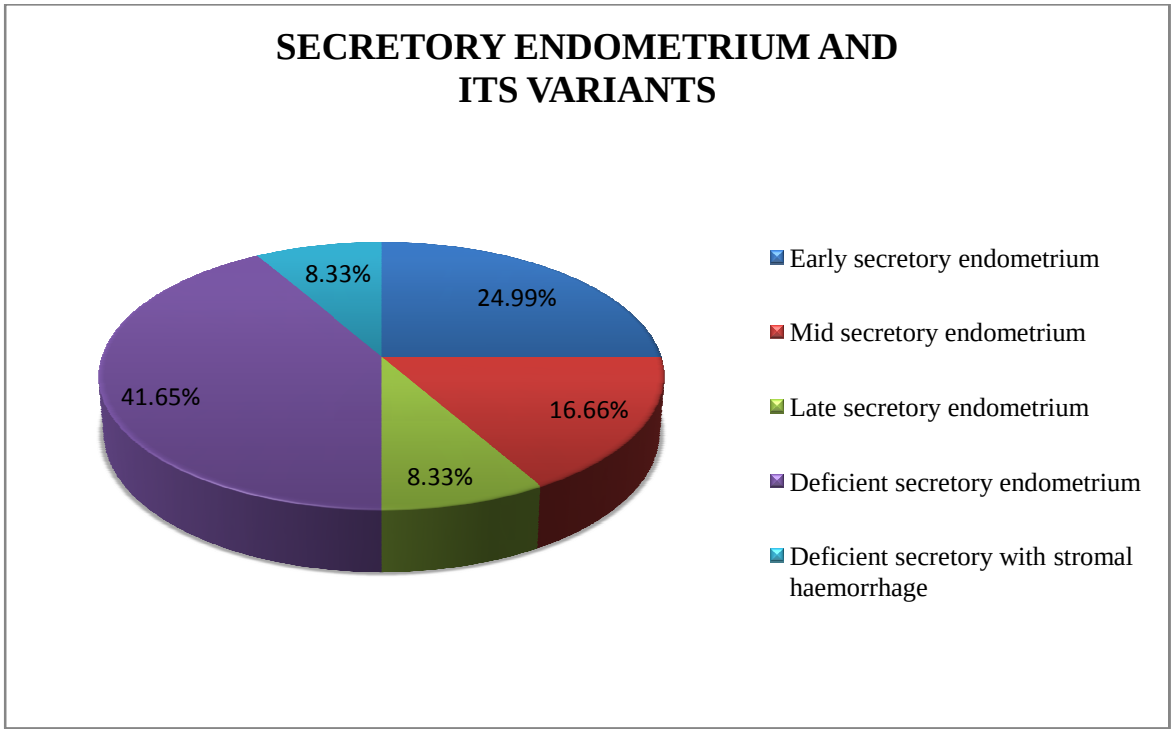


CHART 8

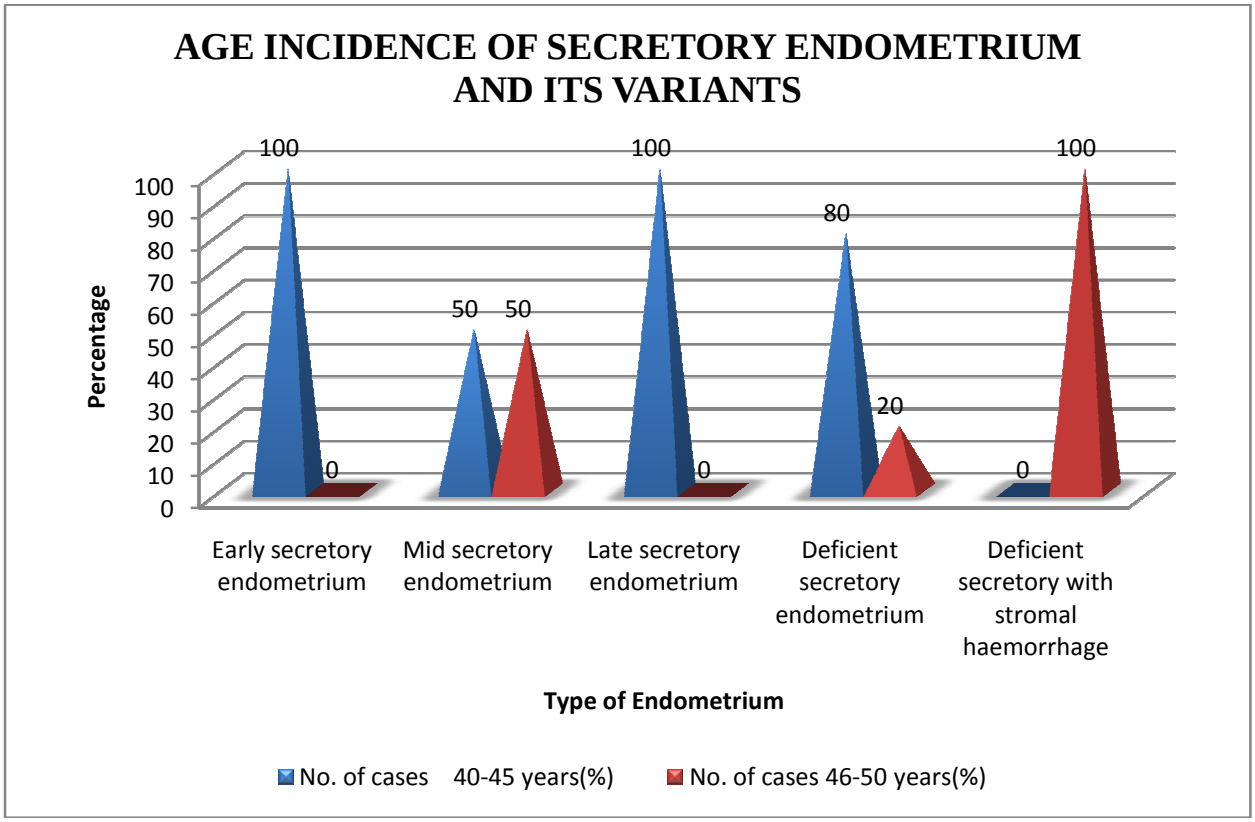


TABLE 15: TYPES OF HYPERPLASIAS

Type of Endometrium	No. of cases	Percentage
TYPICAL		
Simple Hyperplasia without atypia	42	87.50%
Complex Hyperplasia without atypia	4	8.33%
ATYPICAL		
Simple Hyperplasia with atypia	0	0%
Complex Atypical Hyperplasia	2	4.16%
Total	**48**	**100%**

Chi-square with Yate's correction value =7.45, P<0.05.

Among the 48 cases of hyperplasia, in typical hyperplasia, simple hyperplasia without atypia constitute 42 cases (87.50%), complex hyperplasia without atypia constitute 4 cases(8.33%) , and among atypical hyperplasias, complex hyperplasia with atypia was 2 cases (4.16%). Chi-square with Yate's correction test has been applied to find out the association between the hyperplasias in total and those without atypia (typical). The significant p-value indicates that most of the endometrium without atypia (typical)were with simple hyperplasia (Table 15 & Chart 9).

TABLE16: SIMPLE HYPERPLASIA AND ITS VARIANTS

Type of Endometrium	**No. of cases**	**Percentage**
Simple Hyperplasia	29	69.05%
Simple Hyperplasia with disintegration	2	4.76%
Simple Hyperplasia with polyp	2	4.76%
Simple Hyperplasia with chronic endometritis	2	4.76%
Simple Hyperplasia with cystic change	6	14.28%
Simple Hyperplasia with squamous metaplasia	1	2.38%
Total	**42**	**100%**

Among the total 42 cases of simple hyperplasia without atypia,the following patterns were observed.They were, simple hyperplasia without atypia 29 cases (69.05%), simple hyperplasia with disintegration 2 cases (4.76%) simple hyperplasia with polyp 2 cases (4.76%), simple hyperplasia with chronic endometritis 2 cases (4.76%), simple hyperplasia with cystic change 6 cases (14.28%) and simple hyperplasia with squamous metaplasia 1 case (2.38%)(Table 16 & Chart 10).

CHART 9

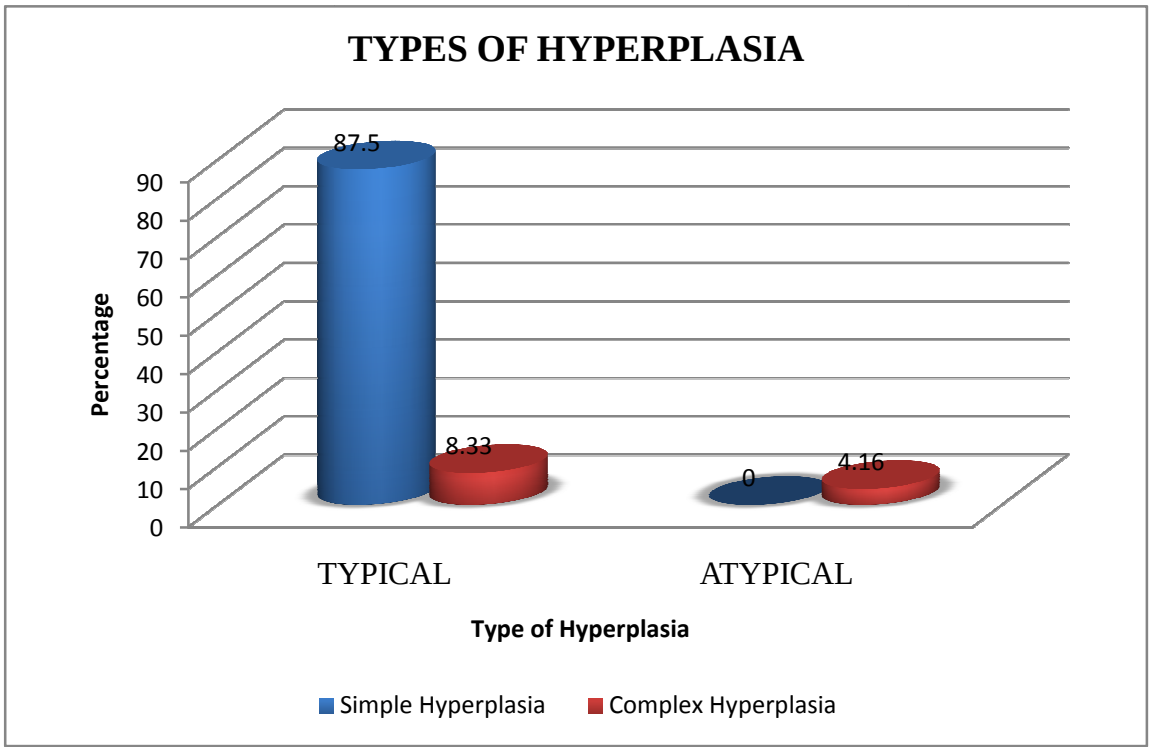


CHART 10

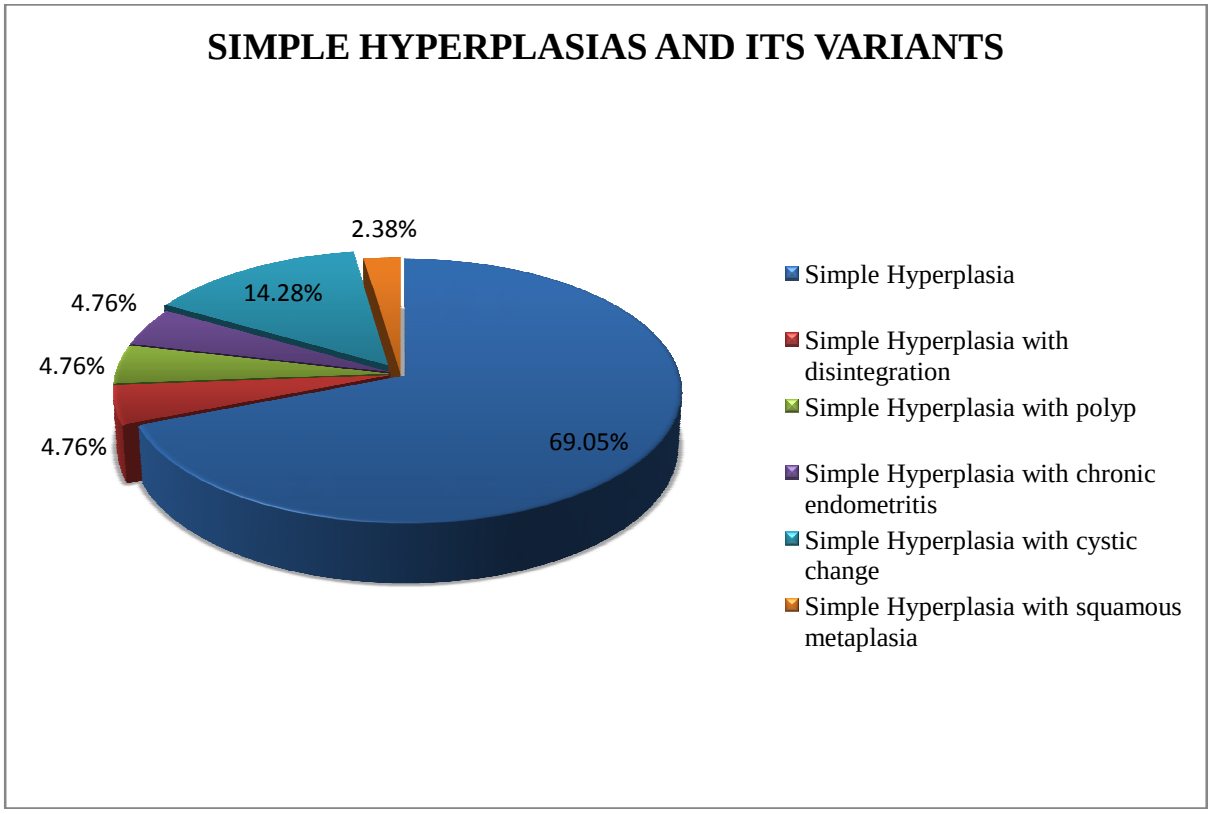


TABLE17: AGE INCIDENCE OF SIMPLE HYPERPLASIA

Type of Endometrium	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Simple Hyperplasia	24	82.7%	5	17.3%
Simple Hyperplasia with disintegration	2	100%	0	0%
Simple Hyperplasia with polyp	2	100%	0	0%
Simple Hyperplasia with chronic endometritis	2	100%	0	0%
Simple Hyperplasia with cystic change	5	83.33%	1	16.66%
Simple Hyperplasia with squamous metaplasia	0	0%	1	100%
Total	35		7	

Chi-square value =1.92, P=0.75(Non-significant).

Between the age group of 40-45 years, there were 35 cases (83.3%), and in the age group of 46-50 years 7 cases (16.66%). Chi-square statistical test has been applied to find out if there is any association between the type of simple hyperplasia and age of the women. The non-significant p-value infers that the type of simple hyperplasia has no association with the age of the women(Table 17& Chart 11).

TABLE18: COMPLEX HYPERPLASIA

Type of Endometrium	No. of cases	Percentage
Complex Hyperplasia without atypia	4	66.67%
Complex Atypical Hyperplasia	2	33.33%
Total	**6**	**100%**

Among the 6 cases of complex hyperplasia, complex hyperplasia without atypia were 4 cases (66.67%), complex hyperplasia with atypia 2 cases (33.33%)(Table 18 & Chart 12).

TABLE19: AGE INCIDENCE OF COMPLEX HYPERPLASIA

Type of Endometrium	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Complex Hyperplasia without atypia	3	75%	1	25%
Complex Atypical Hyperplasia	0	0%	2	100%
Total	**3**		**3**	

Between the age group of 40-45 & 46-50 years, there were 3 cases each of complex hyperplasia without atypia and complex hyperplasia with atypia(Table 19 & Chart 13).

CHART 11

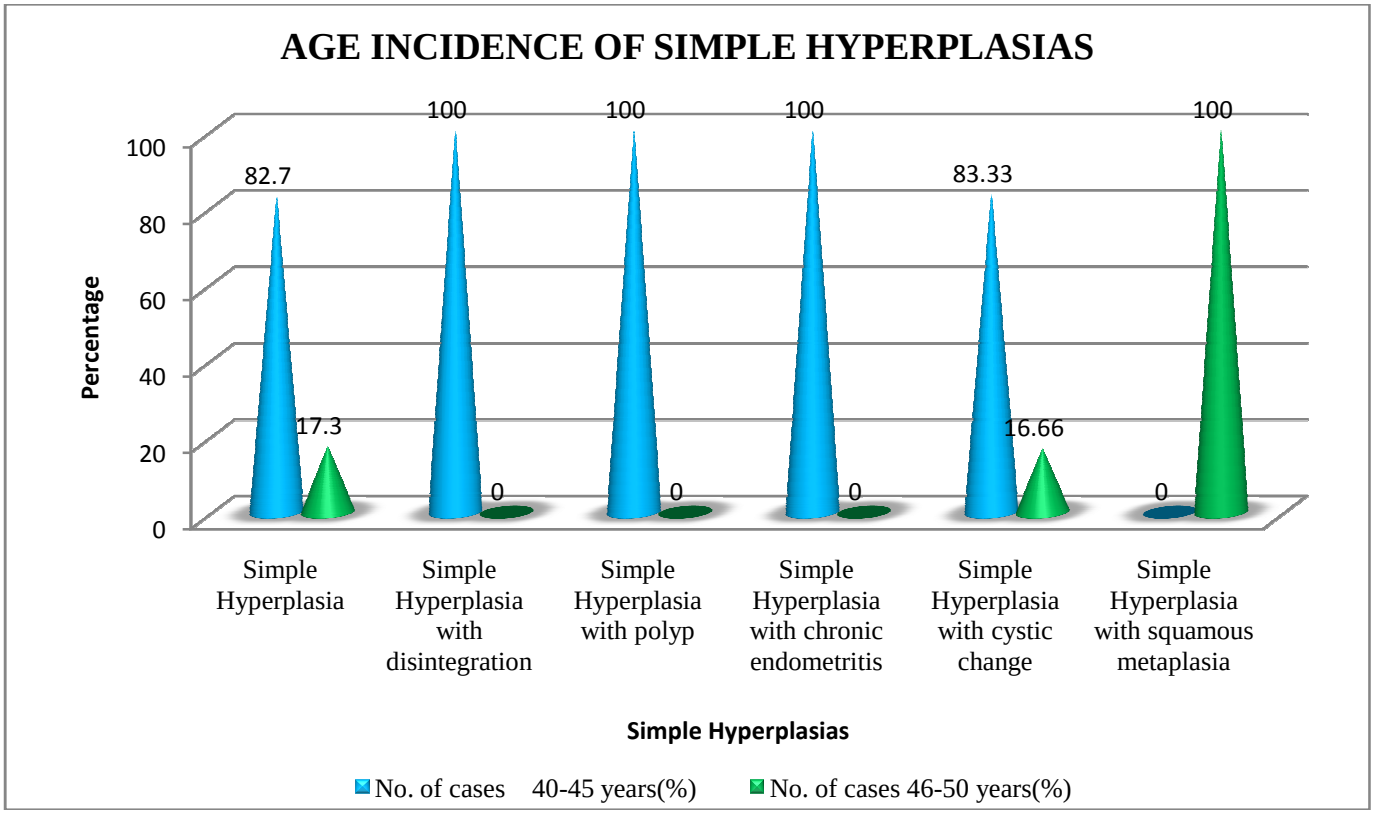


CHART 12

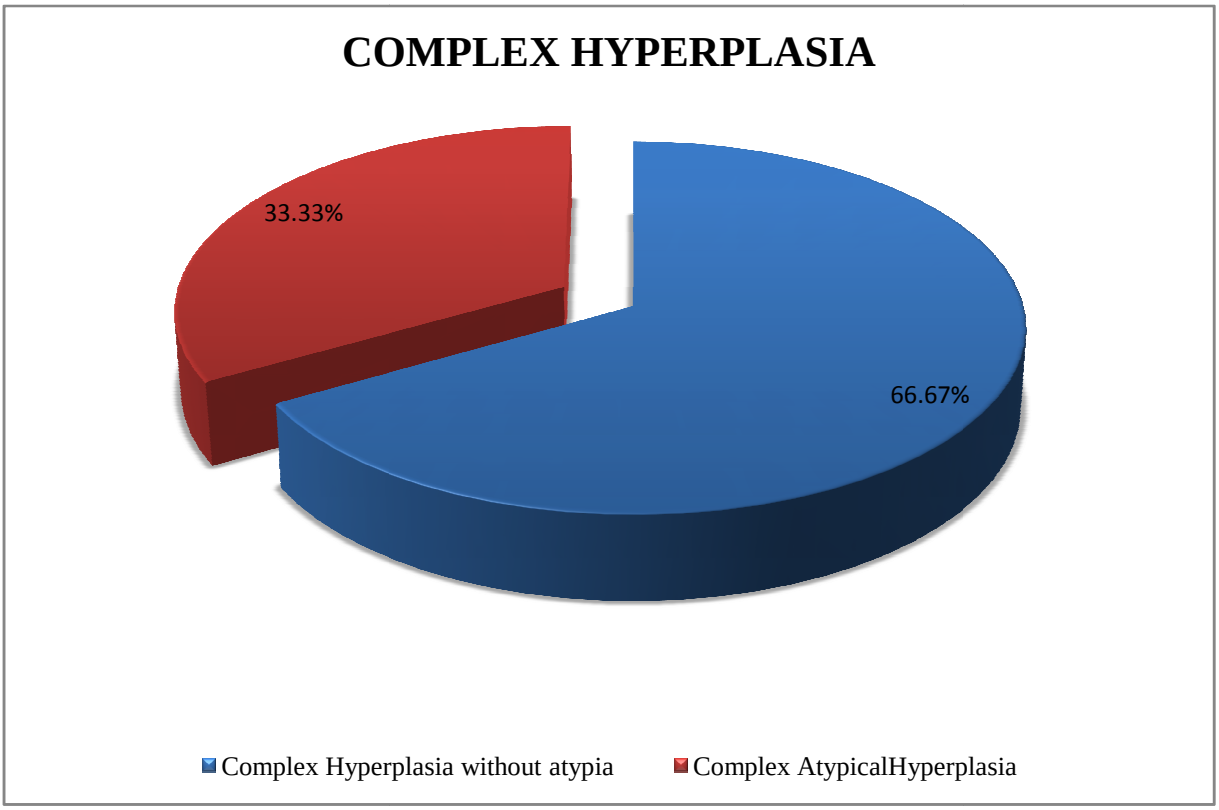


TABLE 20: OTHER PATTERNS OF ENDOMETRIUM

Type of Endometrium	No. of cases	Percentage of Total (200 cases)
Disintegrating endometrium	19	9.5 %
Irregular shedding	16	8%
Endometrial polyp	5	2.5%
Endometrial adeno carcinoma	1	0.5%

The table 20 and Chart 14 depicts the number of cases of disintegrating endometrium 19 cases(9.5%), irregular shedding 16 cases(8%), endometrial polyp 5 cases(2.5%) and endometrial adenocarcinoma 1 case (0.5%).

TABLE 21: AGE INCIDENCE OF OTHER PATTERNS OF ENDOMETRIUM

Type of Endometrium	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Disintegrating endometrium	12	63.16%	7	36.84%
Irregular shedding	11	68.75%	5	31.25%
Endometrial polyp	1	20%	4	80%
Endometrial adeno carcinoma	0	0%	1	100%

CHART 13

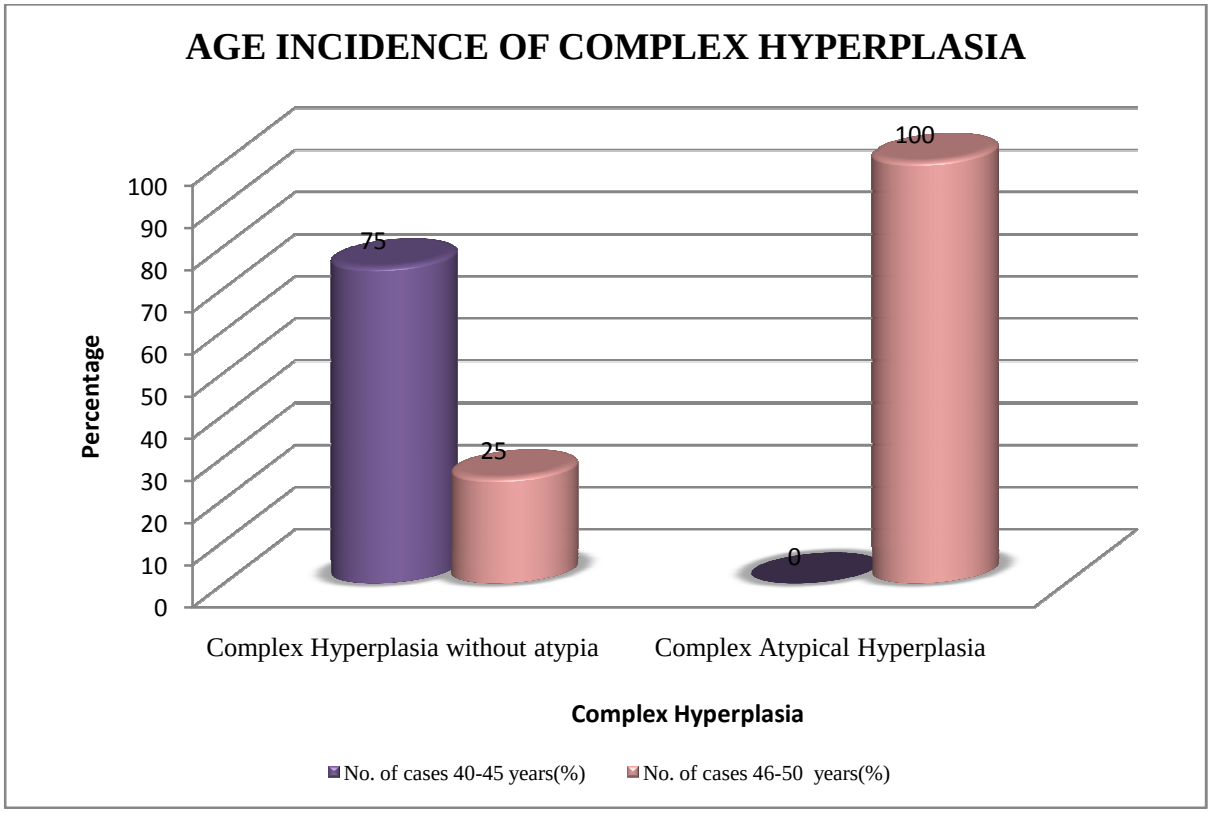
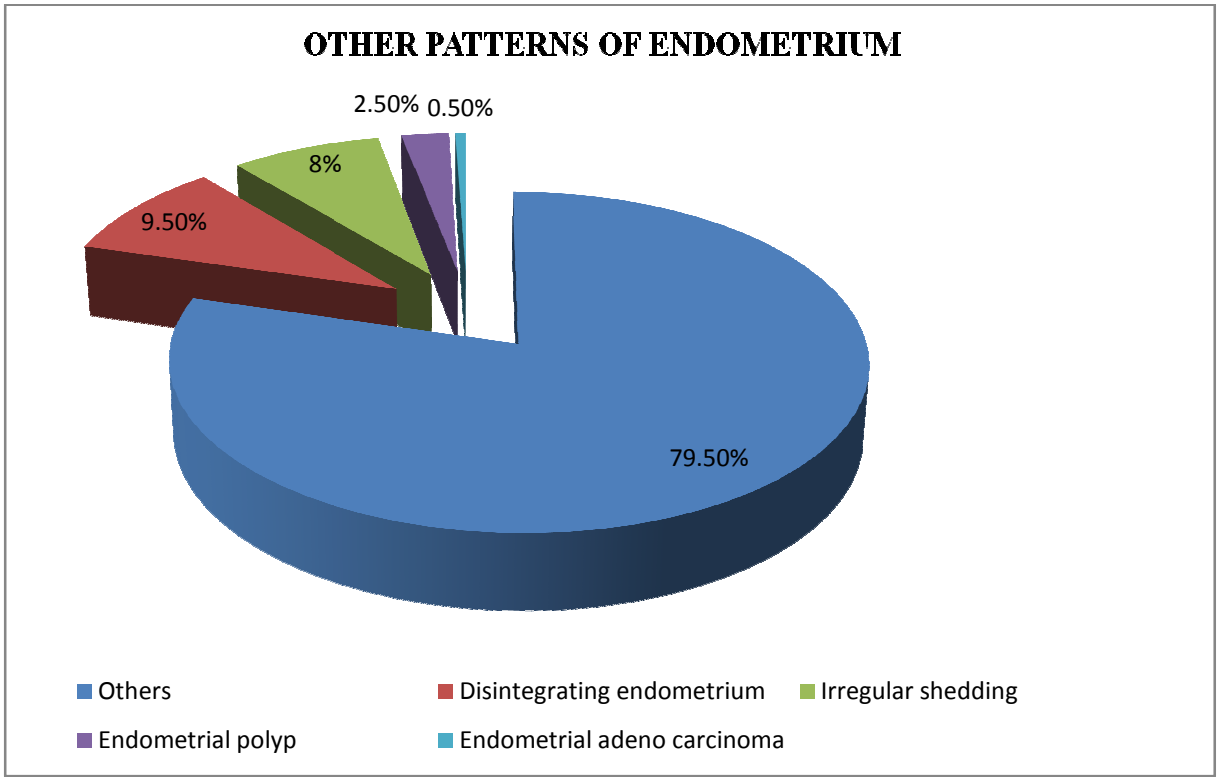


CHART 14



The incidence of disintegrating endometrium in the age group of 40-45 years were 63.16%, irregular shedding 68.75%, endometrial polyp 20% and between 46-50 years, disintegrating endometrium were 36.84%, irregular shedding 31.25%,endometrial polyp 80% and 100% of endometrial adenocarcinoma were observed(Table 21 & Chart 15).

TABLE 22: INCIDENCE OF OTHER ASSOCIATED ORGANIC LESIONS IN PERIMENOPAUSAL AGE

Lesions	**No. of cases**	**Percentage of Total (200 cases)**
Fibroid	37	18.5%
Endometrial polyps	5	2.5%

The table 22 and Chart 16 depicts the other associated organic lesions in perimenopausal bleeding cases. There were 37 cases(18.5%) of fibroid uterus and 5cases(2.5%) of endometrial polyp in the current study.

CHART 15

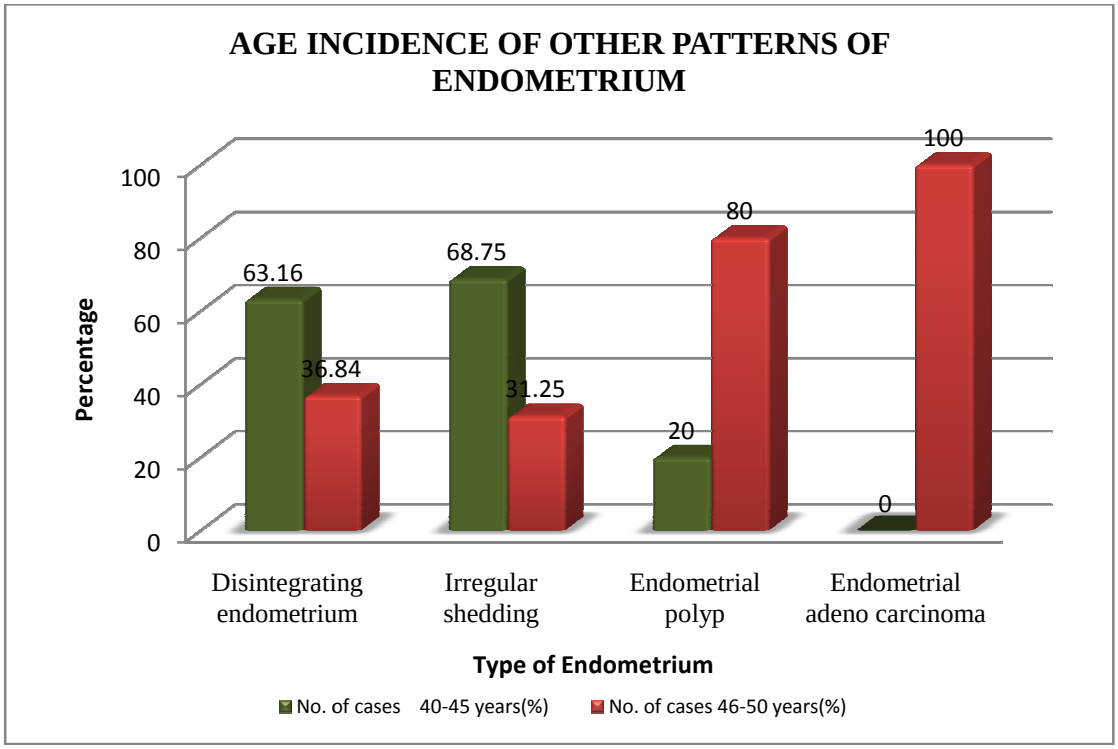


CHART 16

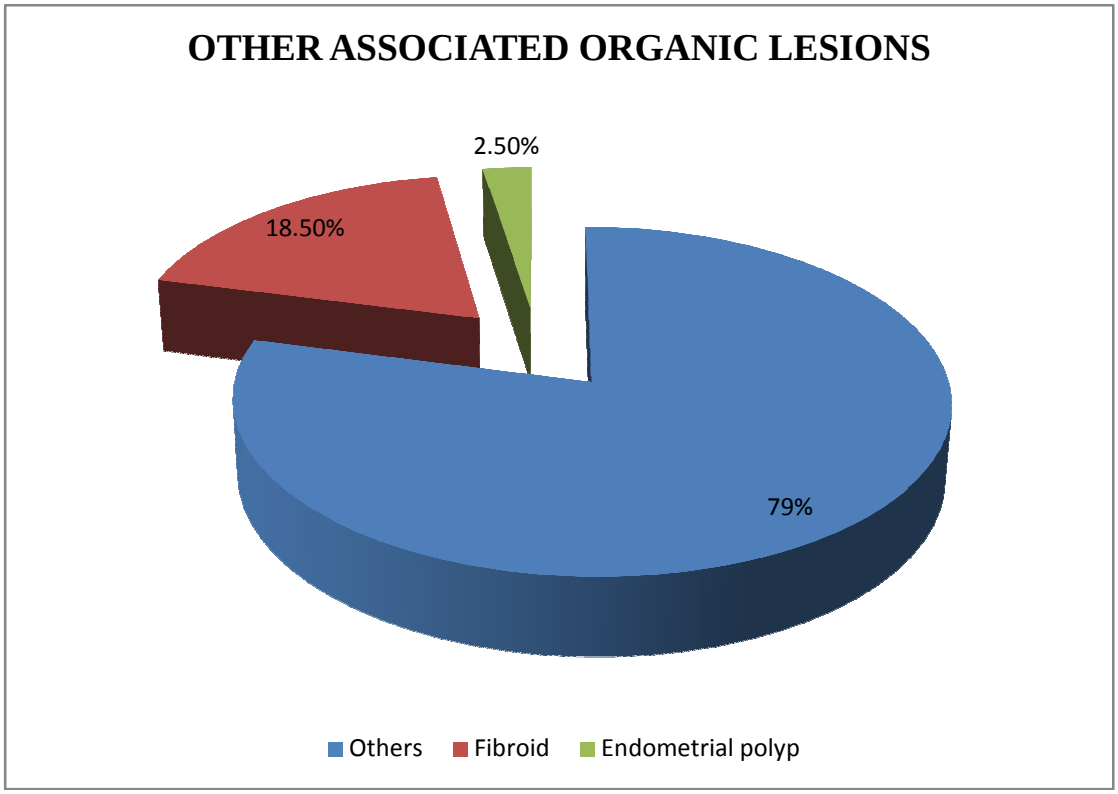


TABLE 23: ENDOMETRIAL PATTERN IN PERIMENOPAUSAL WOMEN WITH FIBROID UTERUS

Type of Endometrium	**No. of cases**	**Percentage**
Proliferative endometrium	5	13.51%
Deficient Proliferative endometrium	3	8.10%
Disordered Proliferative endometrium	1	2.70%
Irregular Proliferative endometrium	1	2.70%
Irregular shedding	4	10.81%
Disintegrating endometrium	8	21.62%
Deficient secretory endometrium	2	5.41%
Simple hyperplasia	12	32.43%
Complex hyperplasia without atypia	1	2.70%
Total	**37**	**100%**

The predominant endometrial pattern observed in women presenting with abnormal uterine bleeding associated with fibroid uterus were simple hyperplasia 12 cases, (32.43%).One case had complex hyperplasia without atypia 2.70% and other patterns were disintegrating endometrium 21.62%, proliferative endometrium 13.51%, deficient

proliferative endometrium 8.10%, deficient secretory endometrium 5.41%, and both disordered proliferative endometrium and irregular proliferative endometrium had similar percentage of 2.70%(Table 23 & Chart 17).

TABLE 24: AGE INCIDENCE OF OTHER ASSOCIATED ORGANIC LESIONS

Lesion	No. of cases 40-45 years	%	No. of cases 46-50 years	%
Fibroid	27	72.97%	10	27%
Endometrial polyp	01	20%	04	80%

The number of cases of perimenopausal bleeding associated with fibroid uterus were higher (72.97%) in 40-45 years age group compared to 46-50 years age group with 27%. Most of the cases(80%)of endometrial polyp were in 46-50 years age group, while in 40-45 years age group 20% were observed(Table 24 & Chart 18).

CHART 17

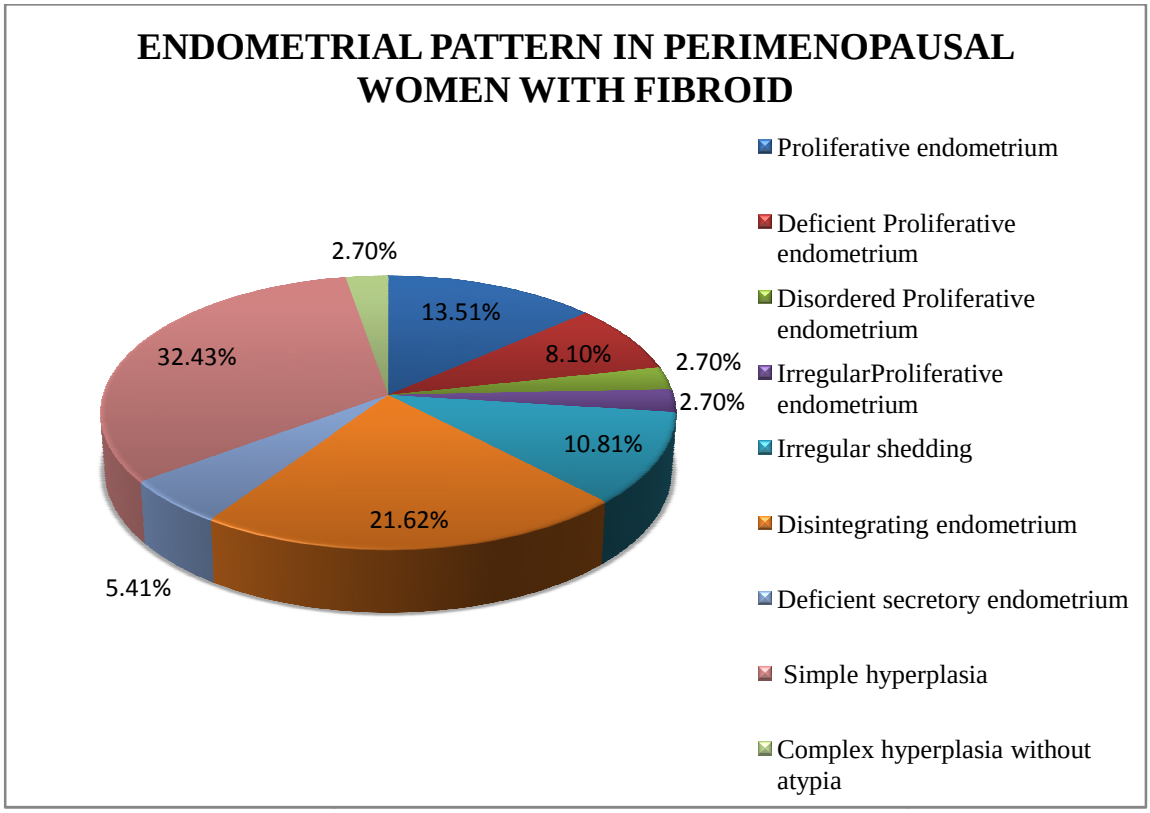
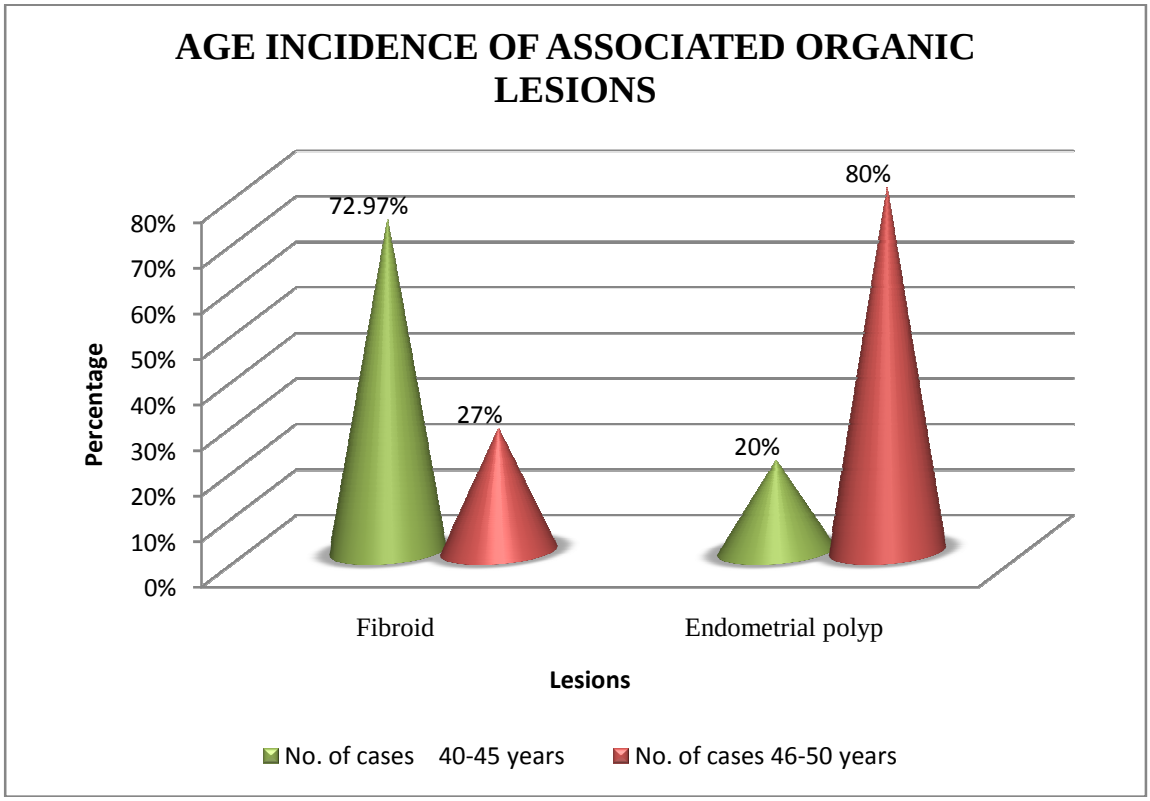


CHART 18



ESTROGEN AND PROGESTERONE RECEPTOR IHC STAINING:

Positive staining of both ER and PR were seen in the nuclei of glands and stroma as fine granular staining. The percentage of cells stained and intensity of reaction were analysed and immunohisto-chemical scoring for estrogen and progesterone receptors were done with Quick score.ER and PR showed nuclear positivity. Staining was graded in the epithelial as well as stromal component in typical and atypical hyperplasias. Malignant epithelium and stroma were also graded for the analysis and calculation of Quick score. Vascular smooth muscle cells and endothelial cells were consistently negative.

Cases of hyperplasias and a case of malignant endometrium evaluated using immunohistochemical method showed the following results

Estrogen Receptor and Progesterone Receptor Staining in Simple Hyperplasia without Atypia:

Simple hyperplasia without atypia showed moderate staining intensity of ER in glands and in the stroma with a mean percentage of positive cells 75% in both the glands and stroma , and moderate staining intensity of PR in glands and in the stroma with a mean percentage of positive cells 75%.

Estrogen Receptor and Progesterone Receptor Staining in Complex Hyperplasia without Atypia:

Complex hyperplasia without atypia showed moderate staining intensity of ER(mean % of positive cells 75%) and PR (mean % of positive cells 85%) in both glands and stroma.

Estrogen Receptor and Progesterone Receptor Staining in Complex Atypical Hyperplasia :

Complex atypical hyperplasia showed moderate staining intensity of ER in glands , weak staining in stromal cells(mean% of positive cells 60%) and moderate intensity levels of PR in both glands and stroma (mean % of positive cells 70%).

Estrogen Receptor and Progesterone Receptor Staining in Neoplastic case:

One case of endometrial adenocarcinoma reported in the current study showed moderate staining intensity of ER in the glands and stroma (mean % of positive cells 45%) and moderate staining intensity of PR in the glands and weak staining in stromal cells (mean % of positive cells 45%).

The percentage of cells stained per 1000 cells counted on 40X power field and the intensity of reaction were analysed and immunohisto -chemical scoring for estrogen and progesterone receptors were done with Quick score which showed the following observations.

TABLE 25: ESTROGEN RECEPTOR EXPRESSION

Type of Endometrium	Glandular cells	Stromal cells
Simple hyperplasia without atypia	Positive	Positive
Complex Hyperplasia without atypia	Positive	Positive
Complex Atypical Hyperplasia	Positive	Positive
Endometrial adenocarcinoma	Positive	Positive

Estrogen receptor expression of all cases were positive in glandular and stromal cells .

TABLE 26: PROGESTERONE RECEPTOR EXPRESSION

Type of Endometrium	Glandular cells	Stromal cells
Simple hyperplasia without atypia	Positive	Positive
Complex Hyperplasia without atypia	Positive	Positive
Complex Atypical Hyperplasia	Positive	Positive
Endometrial adenocarcinoma	Positive	Negative

Progesterone receptor expression in simple hyperplasia without atypia, complex hyperplasias were positive in glandular cells and stromal cells according to Quick score. In endometrial adenocarcinoma, progesterone receptor expression were positive in glandular cells and negative in the stromal cells.

The micro-photographs of some of the slides have been presented in Figures 6-21.

DISCUSSION

Dysfunctional uterine beeding continues to be one of the most frequently encountered problems in Gynaecological practice. In the study period from June 2010 to October 2012, 6583 Gynaecology biopsy specimens were received in Government Medical College, Tirunelveli. Among them, 387 were DUB cases.

TABLE 27: INCIDENCE OF DUB IN VARIOUS STUDY CENTRES

Study Centres	Incidence of DUB (%)
Kamla Nehru Memorial Hospital, Allahabad.[67]	8.32%
AIIMS, New Delhi.[68]	5.3%
Tirunelveli medical college, Tirunelveli	5.88%

The average incidence of DUB cases in our institution was 5.88% as evident from table 27. Similar studies done in other study centres such as Kamla Nehru Memorial Hospital Allahabad, and AIIMS New Delhi showed an incidence of 8.32% and 5.3% respectively.

TABLE 28: INCIDENCE OF PERIMENOPAUSAL BLEEDING CASES

Authors	Total No. of Cases studied	40-50 years	
		No.of cases	Percentage
Sutherland [13] (1950)	1000	362	36.2%
Anusuya Das[69] (1964)	117	38	32.5%
Bhattach arji [70] (1964)	164	44	26.8%
Muzaffar et al[71] (2005)	260	124	48%
Baral R et al [72] (2011)	300	140	46.66%
Current Study	387	200	51.68%

Authors like Sutherland[13] have reported highest incidence of abnormal uterine bleeding in 40-50 years age group, compared to other age groups. Similarly Anusuya Das[69] reported the incidence of abnormal uterine bleeding of 32.5% in the age group of 40-50 years. Bhattacharji (1964)[70] reported highest incidence(46.66) in the age group 40-50 years,while in his study the incidence in other age groups were lower. Muzaffar et al[71] and Baral R et al [72], reported an incidence of 48% and 46.66% respectively. In the current study, the incidence of perimenopausal bleeding was 51.68%.

TABLE 29: DISTRIBUTION OF AGE

Authors	Total No. of cases	40-45 years
Archana Bhosle et al[73]	112	76.1%
Current study	200	67%

Archana Bhosle et al[73]conducted a study for a duration of 6 months on 112 perimenopausal women who presented with abnormal uterine bleeding, of which 76.1% (85 cases) were in 40-45 years age group.In comparision with the above mentioned study, current study also showed higher percentage , 67%(134 cases) in the age group of 40-45 years.

TABLE 30: PATTERNS OF ENDOMETRIUM

Histopathological Diagnosis	Shazia et al(2010)[74]	Layla et al(2011)[75]	Current Study(2013)
Proliferative endometrium and its variants	24%	15.4%	49.5%
Secretory endometrium and its variants	26%	16.6%	6%
Simple(cystic) hyperplasia	25%	10.5%	21%
Complex hyperplasia without atypia	1%	1.8%	2%
Complex atypical hyperplasia	1%	0.54%	1%
Malignancy	0%	1.5%	0.5%

Among the various patterns of endometrium observed,the number of cases of proliferative endometrium and its variants were higher (49.50%) in the present study in comparision to other studies of Layla et al [75]and Shazia et al[74].

TABLE 31: COMPARATIVE ANALYSIS OF HPE OF PROLIFERATIVE ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING CASES[73]

Place	Period	No. of cases	Proliferative Endometrium
Jordan university	Oct-1998-2000	116	53%
LTMMC and Sion hospital	1/5/2007-30/10/2007	112	66.1%
Current study	June 2010-October 2013	200	49.50%

In the present study proliferative endometrium and its variants were higher, (49.50%) similar to the studies conducted in other hospitals as shown in table 31.

Among the cases of proliferative endometrium and its variants in the present study,the disordered proliferative endometrium were higher(26.26%)and the incidence increased with increasing age(46-50 years- 57.7%).This result correlates with the study done by Baral R et al[72].

SECRETORY ENDOMETRIUM:

TABLE 32: INCIDENCE OF SECRETORY ENDOMETRIUM

Authors	**Secretory Endometrium (%)**
Shazia et al[74]	26%
Archana Bhosle et al [73]	16.1%
Layla et al[75]	16.6%
Current study	6%

In the study of Archana Bhosle et al[73] and Layla et al[75] secretory endometrium and its variants were 16%, and the study of Shazia et al [74] had an incidence of 26%. In the present study secretory endometrium and its variants constituted 6%.

Among the cases of secretory endometrium and its variants in the present study,the incidence of early secretory endometrium were 24.99% and the age incidence of secretory endometrium were higher (74.99%)in 40-45 years age group.

ENDOMETRIAL HYPERLASIAS:

TABLE 33: INCIDENCE OF ENDOMETRIAL HYPERPLASIA IN PERIMENOPAUSAL BLEEDING

Authors	Precentage of Cases With Endometrial Hyperlasias
Naheed Moghal[76]	36.84%
Fouzia Adil[77]	41.6%
Present Study	24%

Naheed Moghal[76] studied 114 patients with abnormal uterine bleeding in perimenopausal age group, of which 42 patients had endometrial hyperplasia (36.84%) . In the study of Fouzia Adil[77] he observed that the incidence of cystic hyperplasia and adenomatous hyperplasia were 32.8% was 8.8% respectively.Another study done by Jalil R[78] showed and incidence of 14.42% of endometrial hyperplasia.The incidence of endometrial hyperplasia in the present study was 24% .

SIMPLE HYPERPLASIAS

TABLE 34: COMPARATIVE STUDY OF SIMPLE HYPERPLASIAS OF THE ENDOMETRIUM

Place of study	Period	No. of cases studied	Simple hyperplasia without atypia
LTMMC and Sion hospital[73]	1/5/2007-30/10/2007	112	17.8%
Shazia et al[74]	January to December 2007.	100	25%
Current study	June 2010 to October 2012	200	21%

Of the total 200 cases of perimenoupausal bleeding, the incidence of simple hyperplasia without atypia in the present study were 21% which is in concurrence with the study of Shazia et al[74](25%) and in the study of Archana Bhosle et al [73] 17.8% were observed,as shown in table 34.

In the present study among the 48 cases of endometrial hyperplasias,simple hyperplasia without atypia was most common,with a total of 42 cases (87.50%) similar to the study of Amera Takreem et al[79], who observed 66.6% of simple hyperplasia without atypia in 100 perimenopausal bleeding patients. In our study, 4 cases were complex

hyperplasia without atypia (8.33%) and 2 cases were complex atypical hyperplasia (4.16%). The present study also revealed maximum number of cases of endometrial hyperplasia in the age group of 40-45 years. Simple hyperplasia with atypia which is extremely unusual[40] were not observed in our study.

Complex hyperplasia without atypia were 2% in the present study similar to the study of Layla et al[75].Complex atypical hyperplasia were 1%,similar to the study of Shazia et al [74](1%),and the incidence were observed to be increasing with increasing age(2 cases,46-50 years).

ENDOMETRIAL ADENOCARCINOMA

Lyla et al[75] observed that the incidence of endometrial carcinoma in perimenopausal age group was 1.5%. 1 case (0.5%) of endometrial adenocarcinoma were observed in the present study.

OTHER ASSOCIATED ORGANIC LESIONS:

Fibroid uterus:

In the current study, most of the cases of perimenopausal bleeding with fibroid uterus fall in the age group of 40-45 years(72.97%). The number of perimenopausal bleeding cases associated with fibroid uterus were 37 cases(18.5%) and in the study of Archana Bhosle et al[73] ,it was 49.1%. According to Teleman S et al, 2003,the most frequent pattern of endometrium,in abnormal uterine bleeding was simple hyperplasia.

In the present study, the predominant pattern observed in perimenopausal bleeding cases associated with fibroid uterus is simple hyperplasia 12 cases,(32.43%).One case showed complex hyperplasia without atypia(2.70%),and the other patterns were disintegrating endometrium 21.62%, proliferative endometrium 13.51%, deficient proliferative endometrium 8.10%, deficient secretory endometrium 5.41% and both disordered proliferative and irregular proliferative endometrium were 2.70% .

Endometritis

In the study of Baral R et al[72] the incidence of chronic endometritis was (2.7%) which was equally distributed in reproductive age group and in perimenopausal age.In our study, 2 cases of chronic endometritis were observed.

Endometrial polyps

The prevalence of endometrial polyps in the general population is about 24%. In the study of Baral R et al[72] he observed 4 (1.3%) of the cases having polyp in the perimenopausal age group. In the present study we observed 5(2.5 %) cases of endometrial polyp in which 80% of the cases were in the age group of 46-50 years.

Lyla et al[75] observed that the incidence of endometrial polyp rises as age increases, has a maximum incidence in fifth decade of life and declines gradually after menopause[80].The present study showed an

increasing incidence of endometrial polyps in older age group 46-50 years(80%).Our result is comparable to other studies[81].

ESTROGEN AND PROGESTERONE RECEPTORS:

Hyperplasia in perimenopausal women is a major cause of abnormal uterine bleeding. The transition from complex hyperplasia to carcinoma of endometrium was reported to occur at rates of 26.7% and 29% by Wentz et al and Allahbadia G. et al respectively.This shows the importance of detection of hyperplasias in preventing the disease progression to more advanced stages[82].

Samhita Chakraborty et al (2005) [83]suggested that increased ER and PR lead to a local unopposed estrogen effect. This up regulates estrogen and progesterone receptor protein and this cycle leads to hyperplasia of the endometrium , if the stimulus persists. Endometrial hyperplasia which is a potential precancerous lesion of the endometrium,[84] may show altered expression of sex hormone receptors. Hormonal therapy is an effective treatment strategy in the management of patients with perimenopausal bleeding and it supports the role of these receptors in the etiopathogenesis of hyperplasias[83].

Nyholm et al[84] reported that ER and PR levels were high in simple and complex hyperplasia without atypia, and low in simple hyperplasia with atypia and complex atypical hyperplasia and much lower in adenocarcinoma.

In the study by Daniela et al,[85] of endometrial hyperplasias , 100% of endometrial hyperplasias were positive for stromal and epithelial PR . Daniela et al[85], Samhita Chakraborty et al [83] and Nyholm et al. [84] observed that the mean score of ER and PR decreased in cases of hyperplasias with atypia as compared to hyperplasias without atypia. Samhita Chakraborty et al [83] suggested that the sex steroid receptors may not be the only factor responsible for hyperplasias. There may be other factors responsible for changes leading to atypia and carcinoma, that may down regulate the receptors in atypical hyperplasias.

In the study of Daniela et al(2012)[85], it was observed that the percentage of positive cells for estrogen receptors were 41.50% for simple hyperplasia,72.3% for complex hyperplasia,57% for complex atypical hyperplasia,28.5% for endometrial adenocarcinoma and the percentage of positive cells for progesterone receptors were 43.8% for simple hyperplasia,78.5% for complex hyperplasia,75.4% for complex atypical hyperplasia,29.5% for endometrial adenocarcinoma.

In the current study, the highest values of percentage of positive cells for estrogen receptors (ER)were observed in case of simple hyperplasia without atypia 75% and complex hyperplasia without atypia 75%, followed by complex atypical hyperplasia with 60%. The percentage of positive cells for estrogen receptors in endometrial adenocarcinoma was 45% .

Analyzing the PR expression of various types of endometrial hyperplasia, we observed that complex hyperplasia without atypia has the highest mean values of percentage of positive cells for progestrone receptors (85%), followed by simple hyperplasia without atypia (75%) and complex atypical hyperplasia (70%). The endometrial adenocarcinoma, had a percentage of 45% .

In the present study,we observed that the mean score of ER and PR decreased in cases of hyperplasias with atypia and endometrial adenocarcinoma as compared to hyperplasias without atypia, which is in concurrence with the above mentioned studies of Daniela et al[85], Samhita Chakraborty et al [83] and Nyholm et al [84].After a comparative analysis[85] it was noted that, of ER and PR expression, progesterone receptors are better expressed than estrogen receptors. Thus, for every morphological pattern of the endometrium analyzed in this study, the average values for PR were higher compared with average values of ER for similar lesions.

One case of endometrial adenocarcinoma reported in the current study showed moderate staining of ER and PR in the glands and weak staining in stromal cells. A large array of clinical and pathologic factors have been shown to play significant roles in determining the patient's prognosis in carcinoma of the endometrium[4]. Currently, the steroid hormone receptor status of carcinoma of the endometrium has been demonstrated to be prognostically important.

Both typical hyperplasias and atypical hyperplasias may regress spontaneously over months or few years. However, atypical hyperplasia is a precancerous condition that may progress to malignancy and best treated by surgery with hysterectomy. Hyperplasia without atypia regresses spontaneously after D&C or progestin treatment[42,83]. In patients with atypical hyperplasia, if conserving the uterus is considered, a trial of hormonal treatment may be given[4,86]. Progesterone receptor rich lesions have a better response rate to progestins than lesions which are progesterone receptor poor [56].

Analysis of the steroid hormone receptors play an important role or may be an indication in perimenopausal bleeding patients to predict the response to hormonal therapy[4,83]. Immunohistochemistry explains the response of the patient to hormonal therapy in cases of hyperplasia of endometrium, and suggest that, in these cases, there are many alterations of the cellular DNA, but does not allow the prediction of the cases of atypical hyperplasia which will progress into endometrial carcinoma[85]. In our study, the cases of atypical hyperplasia which are steroid receptor positive,might have responded well if these patients were given hormonal therapy[4].

Immunohistochemical analysis of the Estrogen and Progesterone receptors in hyperplasia of endometrium, and carcinoma of endometrium, allows a more specific determination of the cell ER,PR receptor content and hence yields a more accurate prediction of the response of the patient to endocrine therapy. These studies should facilitate the development of rational strategies for the prevention and treatment of grave and lethal endometrial disorders.

SUMMARY & CONCLUSION

Our study throws a light on many important facts about perimenopausal bleeding

The present study revealed that the incidence of DUB in our institution during the study period is 5.88%, among which, perimenopausal bleeding cases(40-50 years) constituted 51.68%.

The distribution of cases of perimenopausal bleeding was maximum in the age group of 40-45 years(67%) compared to 46-50 years.

The most common histological pattern of endometrium observed was proliferative endometrium and its variants (49.50%).Among hyperplasias, which constituted 24% of the total cases, simple hyperplasia without atypia was higher (87.50%). In the total of 6 cases of complex hyperplasia, 66.67% was complex hyperplasia without atypia and 33.33% complex atypical hyperplasia, and they were equally distributed between the age group of 40-45 & 46-50 years. The other associated organic lesions in perimenopausal age was fibroid uterus 18.5% and endometrial polyps 2.5% and the predominant pattern of endometrium in perimenopausal women with fibroid uterus was simple hyperplasia 32.43%.

Analysing the ER, PR expression by immunohistochemical method revealed that there is significant difference in the epithelial and stromal

expression of PR between simple hyperplasia and complex hyperplasia.Among ER and PR expression, progesterone receptors were better expressed than estrogen receptors. Thus, for every morphological aspect of the endometrium analyzed in this study, the mean values for PR were higher compared with mean values of ER for similar lesions.The cases of atypical hyperplasia which were steroid receptor positive,might have responded well if these patients were subjected to hormonal therapy[4,86]. The response rate to progestins for the lesions which are progesterone receptor rich are better compared to progesterone receptor poor lesions [56].Analysis of the steroid hormone receptors play an important role or may be an indication in perimenopausal bleeding patients to predict the response to hormonal therapy.

The immunohistochemical studies of ER and PR in endometrial hyperplasia and its significance on the prognosis of the disease and hormonal therapy are few in the medical literature compared to receptor studies on breast cancer. Our data will definitely be an important addition to the existing literature.However,further studies have to be conducted in future on a large number of cases for a period of 5-10 years to assess the actual role of estrogen and progesterone receptor expression in Indian population and to explore the possibility of using these receptors as a novel prognostic marker and to predict the response to hormonal therapy.

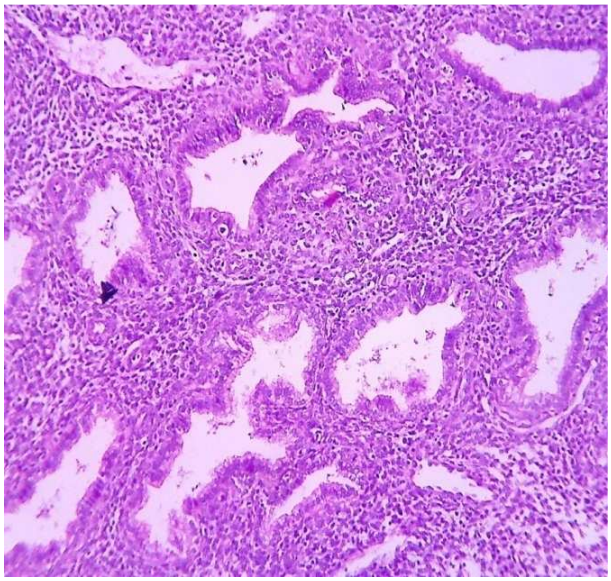


Figure 6:Simple Hyperplasia without atypia(H&E,100X)

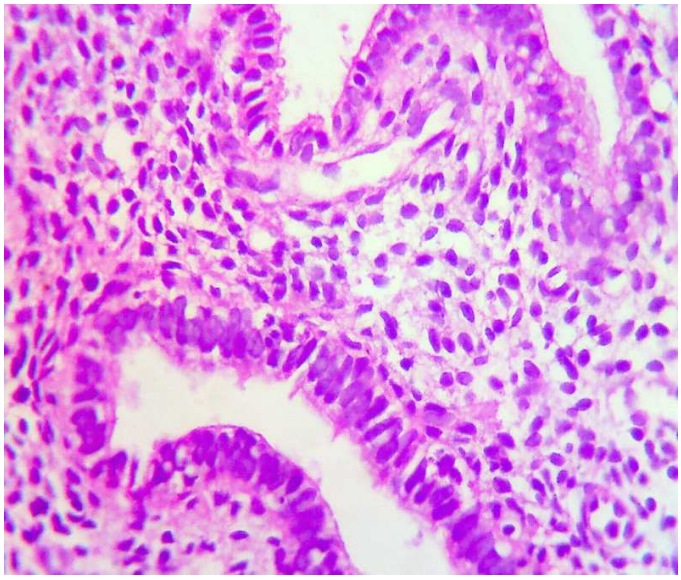


Figure 7:Simple Hyperplasia without atypia(H&E,400X)

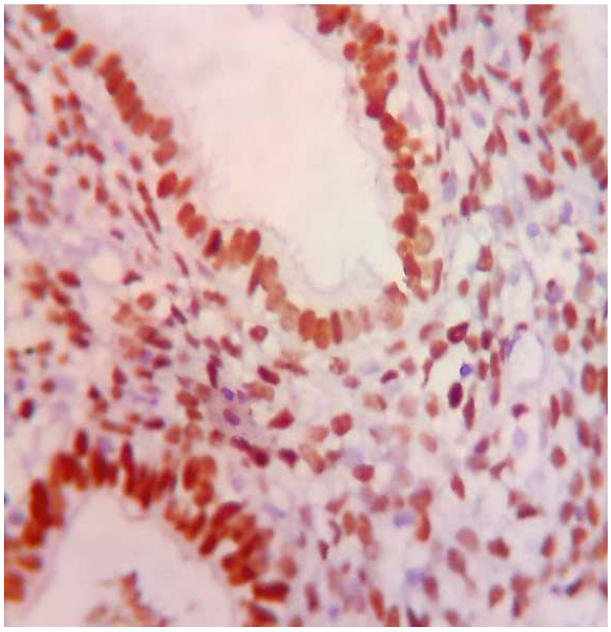


Figure 8: Simple Hyperplasia without atypia(ER,400X)

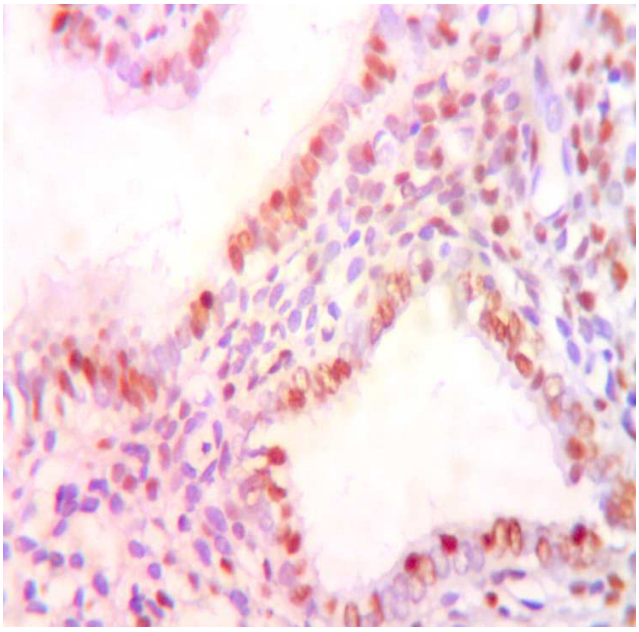


Figure 9: Simple Hyperplasia without atypia(PR,400X)

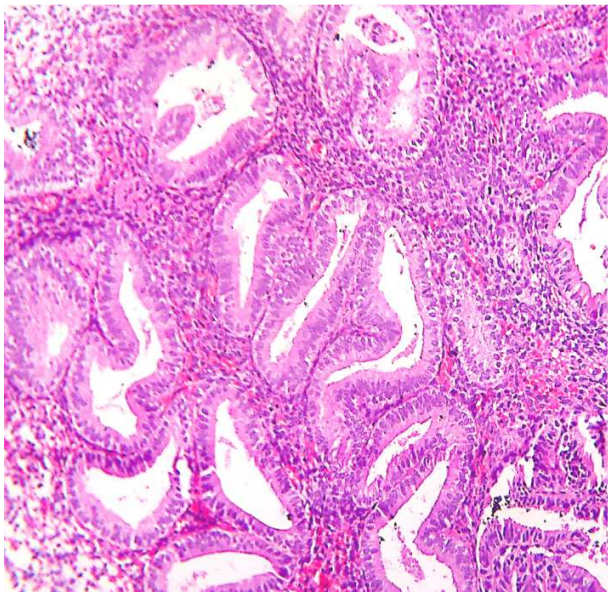


Figure 10:Complex Hyperplasia without atypia(H&E,100X)

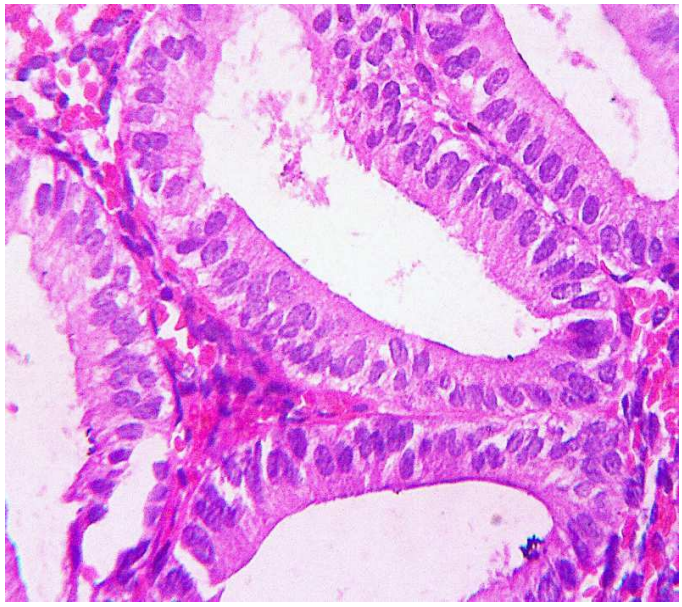


Figure 11: Complex Hyperplasia without atypia(H&E,400X)

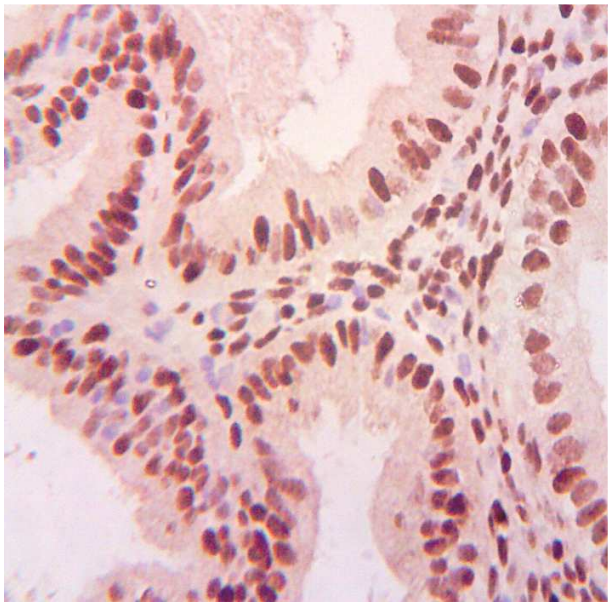


Figure 12: Complex Hyperplasia without atypia(ER,400X)

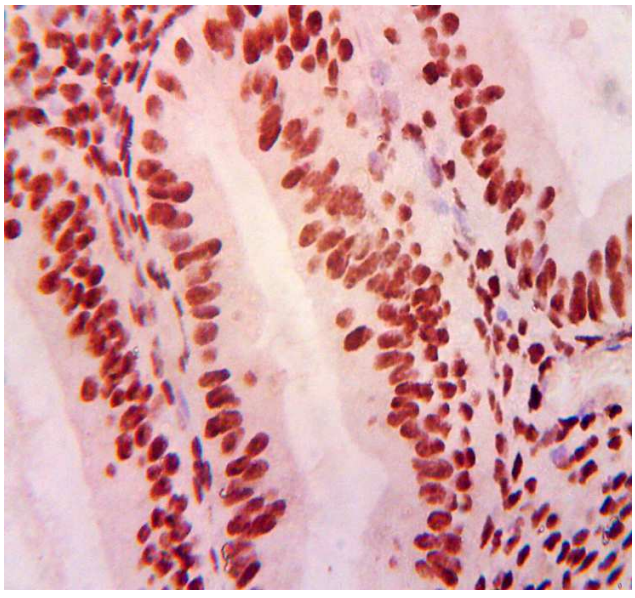


Figure 13: Complex Hyperplasia without atypia(PR,400X)

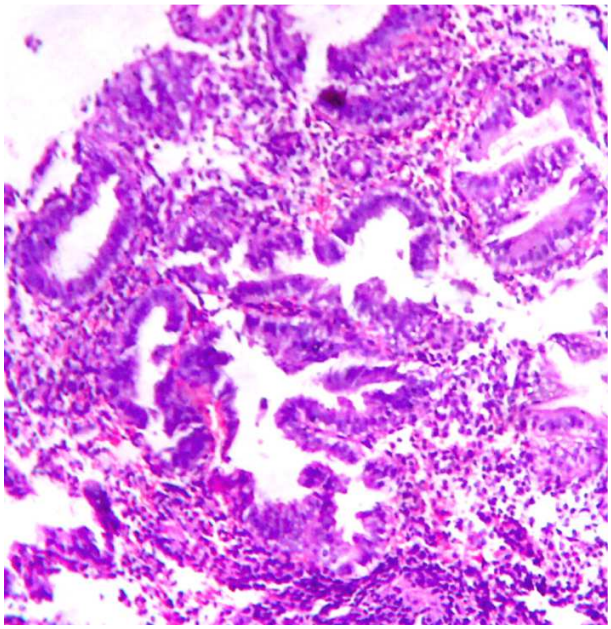


Figure 14:Complex Atypical Hyperplasia (H&E,100X)

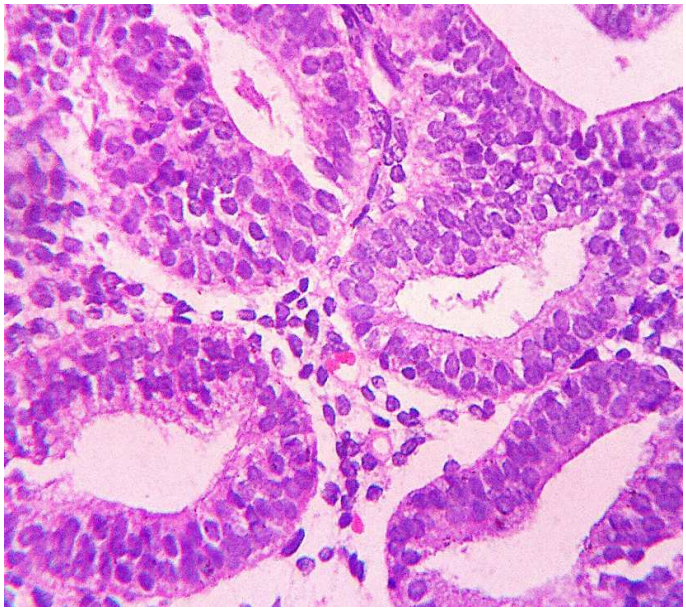


Figure 15:Complex Atypical Hyperplasia (H&E,400X)

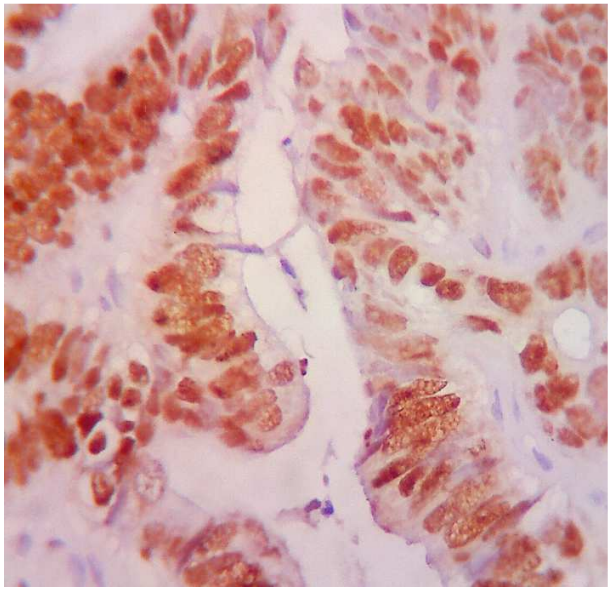


Figure 16:Complex Atypical Hyperplasia(ER,400X)

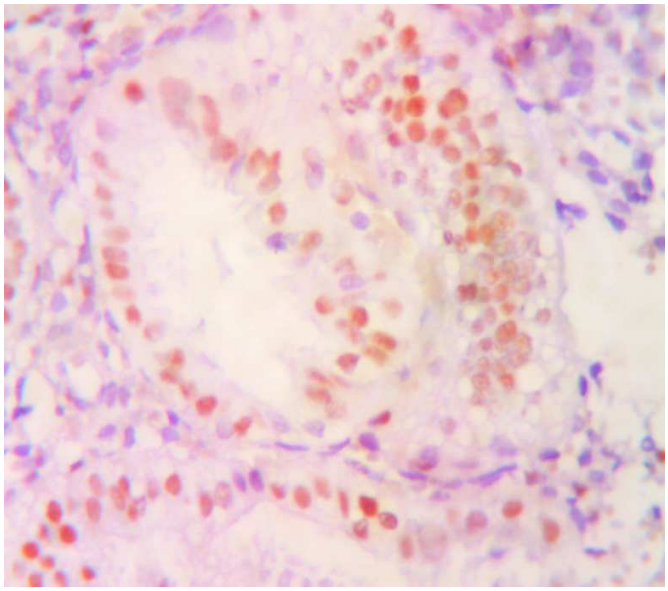


Figure 17:Complex Atypical Hyperplasia(PR,400X)

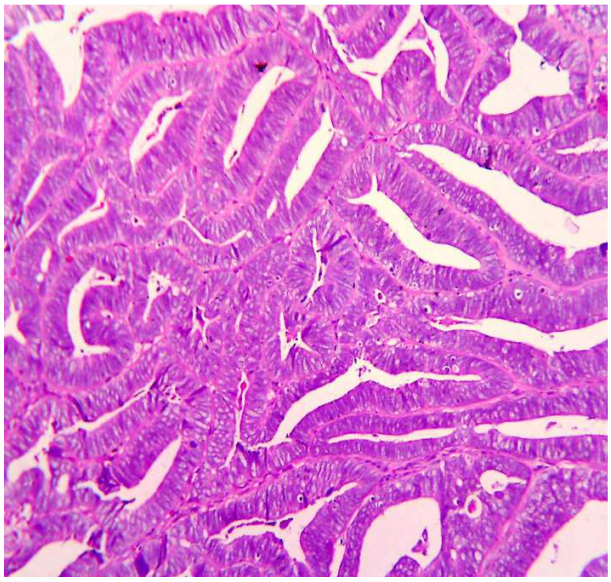


Figure 18: Endometrial adenocarcinoma(H&E,100X)

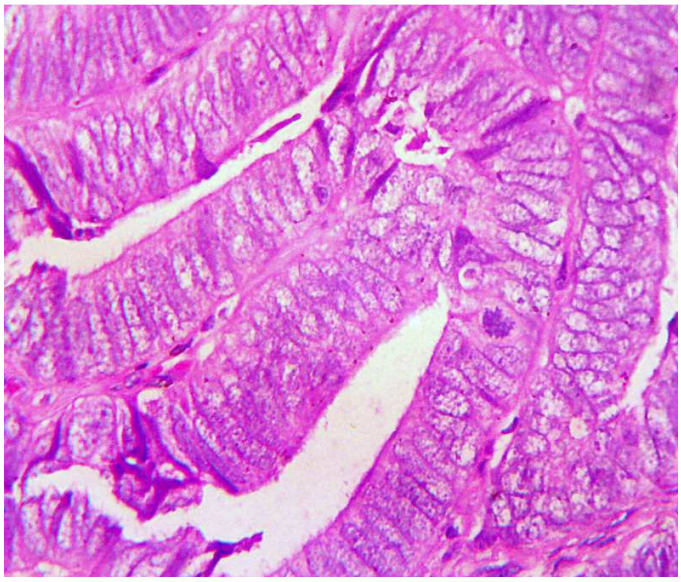


Figure 19: Endometrial adenocarcinoma(H&E,400X)

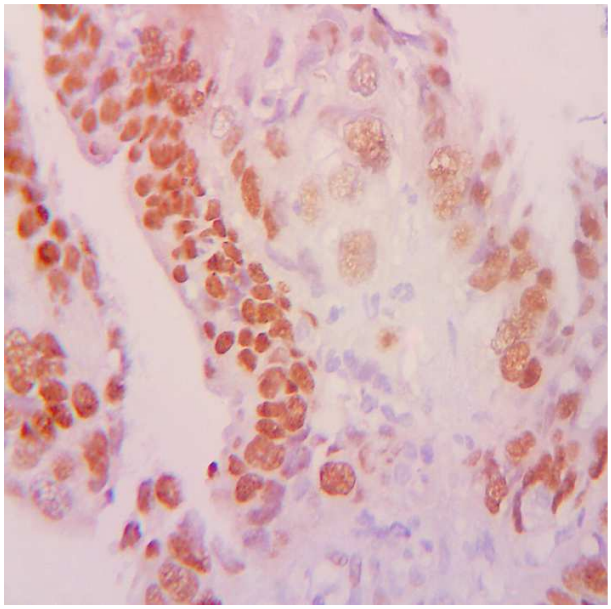


Figure 20: Endometrial adenocarcinoma(ER,400X)

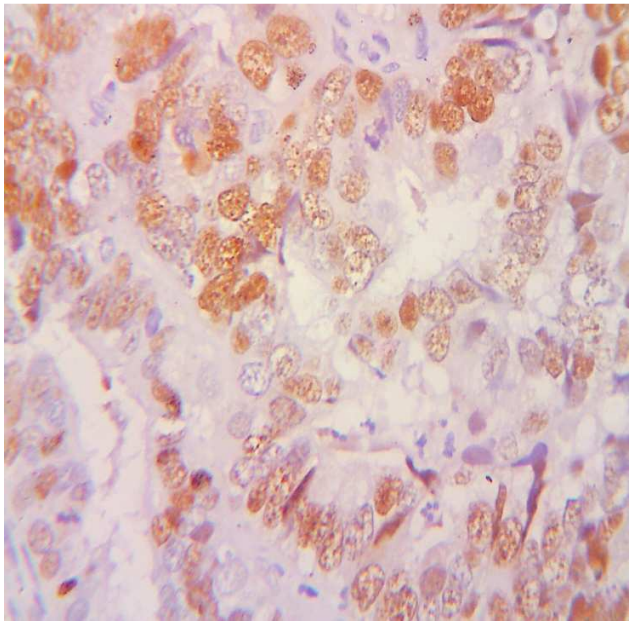


Figure 21: Endometrial adenocarcinoma(PR,400X)

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APPENDIX -1

HAEMATOXYLIN AND EOSIN STAINING PROCEDURE:

1. Dewaxed sections were hydrated through graded alcohols to water.
2. Stained in alum haematoxylin for 7 min.
3. Washed well in running tap water for 5 minutes.
4. Differentiated in acid alcohol for 5 seconds.
5. Washed well in running tap water till the sections are blue for 5minutes.
6. Stained with 1% Eosin Yellow for 3minutes.
7. Washed in running tap water for 5minutes.
8. Dehydrated through alcohols, cleared with xylene and mounted with DPX.

John D.Bancroft, Alan Stevens; "Theory and Practice of Histological Techniques", 4th Ed; Churchill Livingstone; 1996 ;104.

APPENDIX- 2

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections are cut from formalin fixed paraffin embedded tissue samples and are transferred to gelatin-chrome alum coated slides.
2. The slides are incubated overnight at 58ºC .
3. The sections are deparaffinized for 15 minutes x 2 changes, in xylene.
4. The sections are dehydrated for 5 minutes x 2 changes, with absolute alcohol .
5. The sections are washed for 10 minutes in tap water.
6. The slides are then immersed for 5 minutes in distilled water.
7. Using microwave oven, heat induced antigen retrieval was done with appropriate buffer for 20 to 25 minutes, in appropriate temperature .
8. The slides are cooled to room temperature and washed for 5 minutes in running tap water.
9. The slides are then rinsed for 5 minutes in distilled water.
10. Wash the slides for 5 minutes x 2 changes with appropriate wash buffer (phosphate buffer).
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides for 5 minutes x2 changes, in phosphate buffer.

13. The sections are covered with power block for 15 minutes.
14. Appropriate primary antibody is applied over the sections and incubated for 45 minutes, after draining the sections.
15. The slides are covered for 30 minutes with SuperEnhanser.
16. The slides are washed for 5 minutes x2 changes, in phosphate buffer.
17. The slides are covered for 30 minutes with SS Label.
18. Wash the slides for 5 minutes x2 changes, in phosphate buffer.
19. Prepare DAB substrate by diluting 1 drop of DAB chromogen to 1ml of DAB buffer.
20. DAB substrate solution is applied for 8 minutes on the section.
21. Wash the slides for 5 minutes x2 changes, in phosphate buffer.
22. Wash the slides for 5 minutes, in running tap water.
23. Counterstain the sections with Hematoxylin stain for 2 seconds (1 dip).
24. The slides are washed for 3 minutes in running tap water.
25. The slides are air dried,cleared with xylene and mounted using DPX.

APPENDIX- 3

HISTOMORPHOLOGICAL PROFILE OF ENDOMETRIUM IN PERIMENOPAUSAL BLEEDING

PROFORMA

CASE NO: PATH.BIOPSY NO:

PATIENT NAME:

AGE:

IP/ OP. NO. :

UNIT/ WARD :

CLINICAL DIAGNOSIS:

ASSOCIATED ORGANIC LESIONS:

FIBROID / ENDOMETRIAL POLYP.

HPE DIAGNOSIS:

IMMUNOHISTOCHEMICAL STAINING: YES / NO

IHC STAINING DONE : ER / PR / BOTH ER&PR.

INTENSITY OF STAINING:

GLANDS: STROMA: MEAN:

QUICK SCORE FOR INTENSITY:

PROPORTION OF CELLS STAINED:

GLANDS: STROMA: MEAN:

QUICK SCORE FOR PROPORTION :

TOTAL SCORE (INTENSITY+PROPORTION) :

QUICK SCORE RESULT : POSITIVE / NEGATIVE

MASTER CHART

Sl. No.	Biopsy Number	Age (Years)	I.P No.	Clinical Diagnosis	Diagnosis
1	975/10	40	23739	DUB	SHWA
2	990/10	40	23214	DUB	SHWA
3	991/10	41	23409	DUB	DPE
4	1030/10	43	23404	DUB	SHWA
5	1031/10	45	25108	DUB	SHWC
6	1040/10	40	24343	DUB	SHWP
7	1042/10	43	25629	DUB	SHWA
8	1086/10	48	26510	DUB	DPE
9	1087/10	45	26227	DUB	IS
10	1088/10	45	26514	DUB	PE
11	1089/10	50	23905	DUB	SHWS
12	1128/10	45	26049	DUB	DPE
13	1129/10	50	25996	DUB	PEWC
14	1184/10	50	27969	DUB	DE
15	1287/10	43	31750	DUB	SHWA
16	1355/10	40	31523	DUB	SHWA
17	1452/10	42	33880	DUB	DS
18	1453/10	45	32695	DUB	IPE
19	1482/10	43	33242	DUB	DE
20	1499/10	50	34030	DUB	DPE
21	1504/10	47	32583	DUB	MS
22	1536/10	40	36819	DUB	PEWD
23	1538/10	45	34888	DUB	IPE
24	1831/10	48	34939	DUB	DE
25	1877/10	46	37168	DUB	DE
26	1963/10	47	37312	DUB	DPE
27	2088/10	40	42896	DUB	SHWA
28	2156/10	45	44948	AUB(P)	EP
29	2202/10	45	45825	AUB(F)	SHWA
30	2232/10	46	40717	DUB	DPE
31	2240/10	47	46882	AUB(F)	DPE
32	2242/10	47	46894	AUB(F)	DEF.PE
33	2270/10	48	45876	DUB	PEWC
34	2275/10	48	46224	DUB	SHWA
35	2288/10	41	48083	AUB(F)	SHWA
36	2324/10	45	48818	DUB	SHWA
37	2416/10	48	49555	DUB	DPE
38	2417/10	49	50733	AUB(F)	SHWC
39	2418/10	45	51075	DUB	IS
40	2453/10	44	53056	DUB	IS
41	2484/10	44	52739	DUB	DPE
42	2486/10	44	52696	DUB	DPE
43	2488/10	40	52684	DUB	SHWE
44	2502/10	45	533355	DUB	SHWD
45	2505/10	45	51951	DUB	IS
46	2530/10	44	52405	DUB	DPE
47	2633/10	45	56219	DUB	DEF.PE
48	2635/10	48	57590	DUB	DEF.PE
49	2639/10	48	56986	DUB	DEF.PE

50	2640/10	45	57097	DUB	DEF.PE
51	20/11	43	56261	DUB	PEWD
52	26/11	44	54972	DUB	CHWA
53	28/11	40	58315	DUB	DEF.PE
54	29/11	42	58304	DUB	IPE
55	33/11	46	645	DUB	IS
56	62/11	42	58182	DUB	DS
57	71/11	40	1725	DUB	IPE
58	72/11	48	974	DUB	IS
59	102/11	45	968	DUB	DEF.PE
60	103/11	41	2744	DUB	DEF.PE
61	113/11	48	3936	DUB	DEF.PE
62	115/11	44	3096	DUB	DEF.PE
63	168/11	46	2560	DUB	IPE
64	173/11	47	3964	DUB	DEF.PE
65	187/11	42	4529	DUB	DEF.PE
66	189/11	40	4829	DUB	IS
67	195/11	44	5034	DUB	CHWA
68	226/11	40	1795	AUB(F)	DS
69	228/11	41	5610	DUB	PEWD
70	229/11	50	5220	DUB	DPE
71	267/11	46	7697	DUB	IS
72	268/11	45	3284	DUB	ES
73	283/11	45	7297	AUB(F)	DEF.PE
74	311/11	44	7656	AUB(F)	SHWC
75	325/11	43	8566	AUB(F)	DS
76	365/11	45	9479	DUB	IS
77	374/11	48	9631	DUB	DPE
78	377/11	50	9449	DUB	DE
79	432/11	44	12506	DUB	PEWD
80	437/11	50	11148	DUB	DEF.PE
81	455/11	45	11870	DUB	IPE
82	461/11	40	12111	DUB	SHWE
83	662/11	40	1204	AUB(F)	SHWA
84	680/11	40	11810	DUB	DEF.PE
85	687/11	43	12827	DUB	PE
86	716/11	49	12593	AUB(F)	DE
87	730/11	49	14281	DUB	DEF.PE
88	768/11	45	14636	DUB	DEF.PE
89	824/11	42	16905	DUB	PE
90	859/11	45	14159	DUB	SHWC
91	888/11	45	17465	DUB	PEWC
92	945/11	47	19640	DUB	SHWA
93	998/11	43	21059	DUB	DPE
94	1034/11	44	21678	AUB(F)	SHWA
95	1036/11	40	22089	AUB(F)	PE
96	1039/11	50	21150	AUB(P)	EP
97	1043/11	43	23251	DUB	CHWA
98	1102/11	49	22305	AUB(P)	EP
99	1105/11	40	23087	DUB	SHWP
100	1143/11	49	24203	AUB(P)	EP
101	1144/11	45	25056	AUB(F)	SHWA

102	1337/11	45	26575	AUB(F)	DEF.PE
103	1361/11	40	27161	DUB	SHWA
104	1362/11	48	28433	DUB	DS
105	1363/11	42	27975	DUB	PEWD
106	1393/11	47	27539	DUB	DPE
107	1431/11	42	28136	DUB	DPE
108	1433/11	41	28927	DUB	PEWD
109	1462/11	45	29255	DUB	DPE
110	1482/11	47	30509	DUB	DPE
111	1511/11	43	30501	AUB(F)	DE
112	1542/11	40	31702	AUB(F)	DE
113	1551/11	45	33311	DUB	DPE
114	1553/11	42	32727	AUB(F)	DE
115	1643/11	48	34517	DUB	DPE
116	1647/11	40	34512	DUB	MS
117	1756/11	45	36021	AUB(F)	PE
118	1757/11	44	38223	DUB	DPE
119	1779/11	45	34722	DUB	DE
120	1801/11	42	38261	DUB	DE
121	1802/11	46	38397	DUB	DPE
122	1842/11	50	40988	DUB	CAH
123	1861/11	42	39223	DUB	DE
124	1888/11	50	40511	DUB	PE
125	1889/11	50	39392	DUB	DE
126	1894/11	40	41633	DUB	LS
127	1898/11	45	41006	AUB(F)	DE
128	1899/11	45	40139	DUB	DPE
129	1908/11	45	41182	DUB	PE
130	1957/11	46	41821	DUB	DPE
131	2095/11	40	45281	AUB(F)	SHWA
132	2139/11	44	45084	DUB	ES
133	2166/11	48	46026	DUB	PEWD
134	2231/11	45	47071	DUB	SHWC
135	2260/11	50	47303	DUB	DEF.PE
136	2316/11	40	50052	AUB(F)	SHWC
137	2379/11	50	50631	DUB	SHWA
138	2382/11	43	51402	DUB	SHWA
139	2403/11	40	53434	AUB(F)	IS
140	2404/11	40	51987	AUB(F)	SHWA
141	2410/11	49	50908	DUB	SHWA
142	2411/11	46	53596	DUB	DEF.PE
143	2432/11	45	53229	DUB	DEF.PE
144	2447/11	43	53040	DUB	PEWD
145	2450/11	41	54742	DUB	SHWA
146	2460/11	46	52624	DUB	DSSH
147	2486/11	40	54446	DUB	PE
148	2548/11	45	55559	DUB	SHWD
149	2551/11	40	55908	AUB(F)	PE
150	2572/11	50	54883	DUB	DPE
151	004/12	49	60926	AUB(P)	EP
152	100/12	47	50989	AUB(F)	IS
153	130/12	42	2187	AUB(F)	IPE

154	176/12	50	1721	DUB	IPE
155	182/12	45	2820	DUB	PE
156	185/12	40	3265	AUB(F)	IS
157	226/12	43	2931	DUB	DEF.PE
158	309/12	47	5833	DUB	PEWD
159	373/12	40	6694	DUB	PE
160	375/12	45	6733	AUB(F)	PEWD
161	535/12	45	9989	AUB(F)	IS
162	676/12	41	10416	DUB	IPE
163	723/12	44	13835	DUB	ES
164	727/12	42	16283	DUB	PE
165	730/12	42	15228	DUB	SHWA
166	746/12	44	13889	DUB	PE
167	748/12	44	16680	DUB	PE
168	749/12	43	16743	DUB	PE
169	834/12	43	19269	DUB	PE
170	866/12	46	17401	DUB	CAH
171	870/12	45	16288	DUB	PE
172	920/12	40	22337	DUB	IS
173	981/12	48	21442	AUB(F)	SHWA
174	1009/12	42	23585	DUB	SHWA
175	1139/12	50	27157	DUB	DEF.PE
176	1150/12	48	28390	DUB	DPE
177	1215/12	40	31888	AUB(F)	SHWA
178	1216/12	43	30128	DUB	SHWA
179	1222/12	41	30411	DUB	PE
180	1254/12	50	25915	DUB	EACA
181	1255/12	40	23860	DUB	SHWA
182	1391/12	45	35319	DUB	PE
183	1404/12	45	36605	DUB	IS
184	1409/12	47	36537	DUB	IS
185	1424/12	43	37020	DUB	PE
186	1444/12	45	36608	DUB	DE
187	1478/12	45	38429	DUB	PE
188	1507/12	50	39516	AUB(F)	CHWA
189	1585/12	48	40907	AUB(F)	PE
190	1586/12	49	40294	AUB(F)	DE
191	1635/12	48	43161	DUB	IPE
192	1652/12	42	44605	DUB	IPE
193	1659/12	41	44756	DUB	SHWA
194	1696/12	45	44201	DUB	DE
195	1728/12	48	44719	DUB	IPE
196	1729/12	49	46060	AUB(F)	DE
197	1730/12	41	44756	DUB	PE
198	1732/12	45	47215	AUB(F)	DE
199	1862/12	49	49904	DUB	DEF.PE
200	1957/12	40	51819	DUB	DEF.PE

KEY TO MASTER CHART

AUB	:	Abnormal uterine bleeding
AUB(P)	:	Abnormal uterine bleeding with polyp
AUB(F)	:	Abnormal uterine bleeding with fibroid
CAH	:	Complex atypical hyperplasia
CHWA	:	Complex hyperplasia without atypia
DE	:	Disintegrating endometrium
DEF.PE	:	Deficient Proliferative endometrium
DPE	:	Disordered Proliferative endometrium
DS	:	Deficient secretory
DSSH	:	Deficient secretory with stromal hemorrhage
DUB	:	Dysfunctional uterine bleeding
EACA	:	Endometrial adenocarcinoma.
EP	:	Endometrial polyp
ES	:	Early secretory endometrium
IPE	:	Irregular proliferation of endometrium
IS	:	Irregular shedding
LS	:	Late secretory endometrium
MS	:	Mid secretory endometrium
PE	:	Proliferative endometrium
PEWC	:	Proliferative endometrium with cystic change
PEWD	:	Proliferative endometrium with disintegration

SHWA	:	Simple hyperplasia without atypia
SHWC	:	Simple hyperplasia with cystic change
SHWD	:	Simple hyperplasia with disintegration
SHWE	:	Simple hyperplasia with chronic endometritis
SHWP	:	Simple hyperplasia with polyp
SHWS	:	Simple hyperplasia with squamous metaplasia